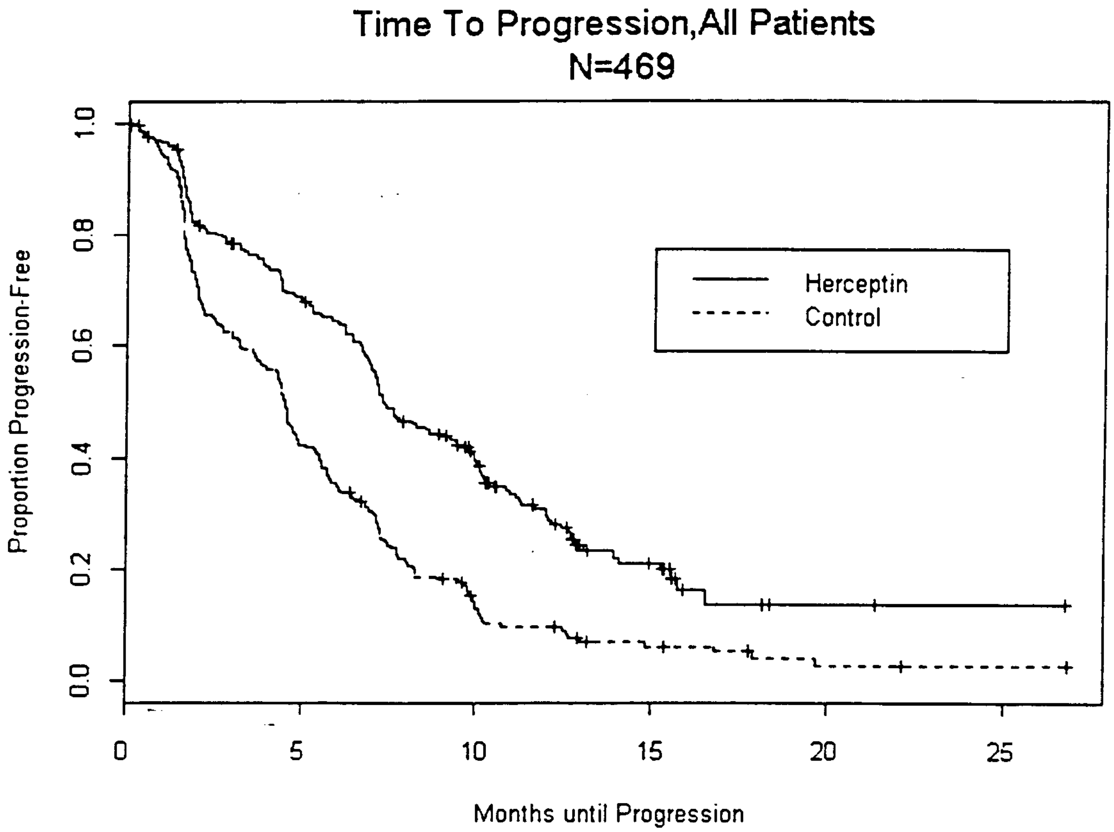

Figure 2. Time to progression comparison for the two study arms: Herceptin® plus chemotherapy (Herceptin) and chemotherapy alone (control). Study H0648g.

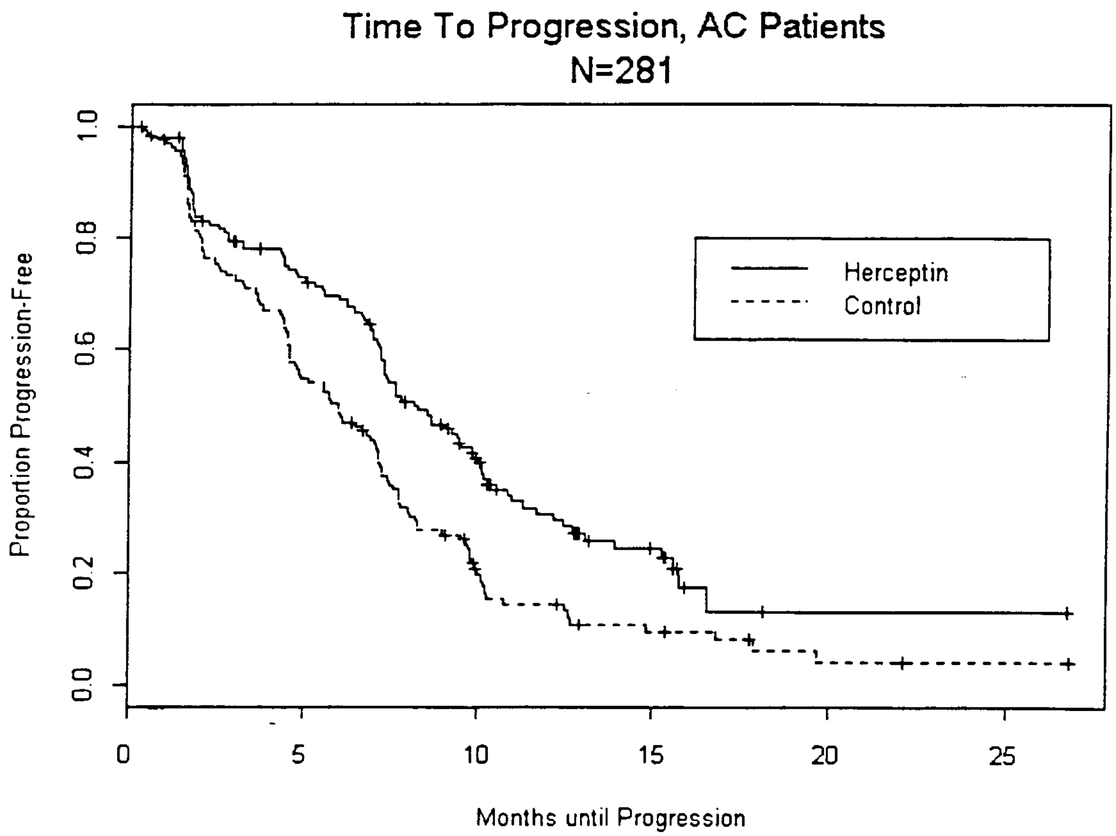

Figure 3. Time to progression comparison of the two AC subgroups: AC plus Herceptin® (Herceptin) vs. AC alone (control). Study H0648g.

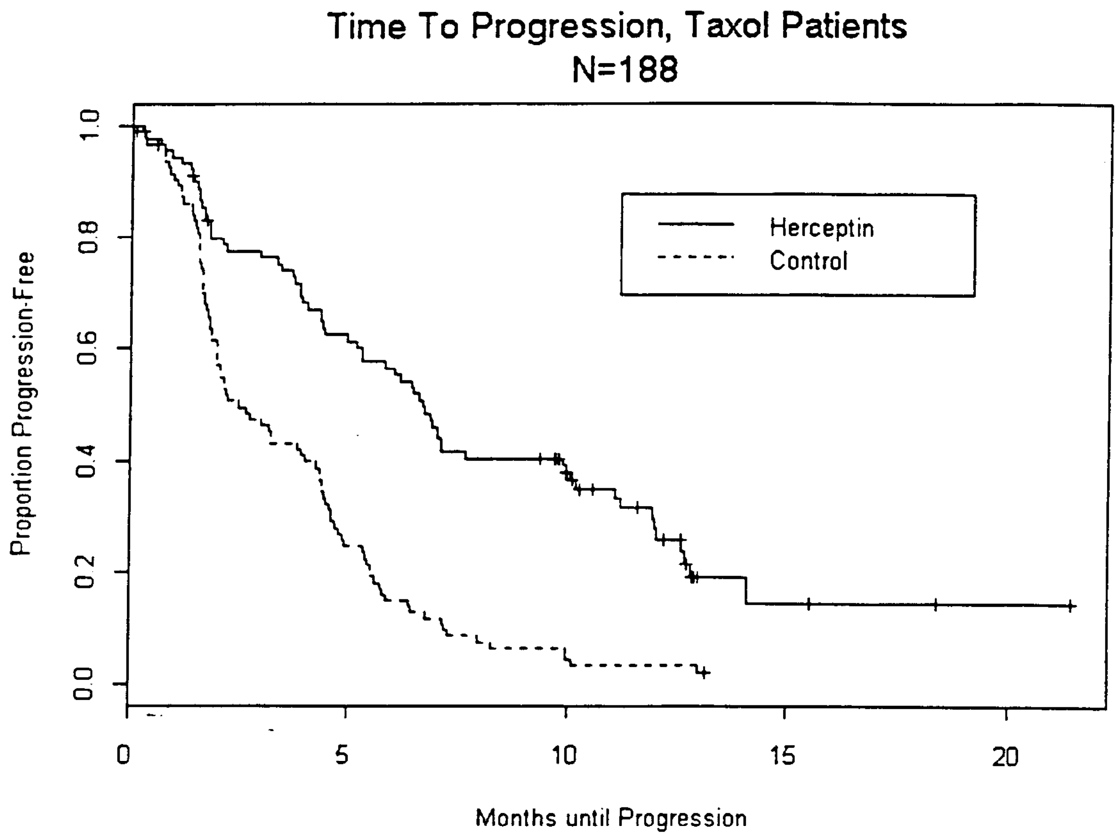

Figure 4. Time to progression comparison of the two paclitaxel subgroups: Herceptin® plus T (Herceptin) vs. T alone (control). Please note that the x-axis scale is different than in Figure 3; this will be corrected. Study H0648g.

6.6.9 Secondary Efficacy Endpoints

Response Rate

The overall response rate was prospectively defined by the protocol to be the sum of the complete and partial response rates, sustained for 4 or more weeks as determined by the REC. The FDA analyzed the response data as submitted in the SAS data sets by the sponsor. The FDA analysis of the data included only the REC confirmed responders. We has asked the sponsor to have the REC read all films for all patients whom they had not evaluated at the time of BLA filing, regardless of their investigator determined progression status. The decision by the FDA to request the data in this manner rested on the fact that investigator tumor measurements were not collected on the data forms and there was no mechanism for the FDA to confirm the investigator responses.

The response rate data appear in Tables 17 and 18. Patients receiving Herceptin® plus chemotherapy had a higher response rate than those receiving chemotherapy alone: 45% vs 29%, respectively. This effect was seen primarily in the paclitaxel subgroup: for ACH vs AC groups the response rates were 50% vs 38% ($p = 0.10$), respectively and for the TH vs T groups they were 38% vs 15% ($p = 0.001$), respectively. The median duration of response was also longer for the Herceptin® treated patients: 2.7 months longer for the ACH group vs AC and 4.0 months longer for the TH group vs T alone.

Table 17. Response rate and duration of response - Sponsor derived data sets H0648g

Parameter	ACH n, (%) n = 143	AC n, (%) n = 138	TH n, (%) n = 92	T n, (%) n = 96	H + Chemo n, (%) n = 235	Chemo alone n, (%) n = 234
All responses (CR + PR)	70 (49)	65 (47)	32 (35)	25 (26)	102 (43)	90 (38)
All responses (CR)	5 (3)	7 (5)	4 (4)	2 (2)	9 (4)	9 (4)
Confirmed responses (REC)	51 (36)	49 (36)	20 (22)	12 (13)	71 (30)	61 (26)
Median Duration of response for confirmed responses (mos)	5.7	5.6	6.3	4.3	5.7	5.5
95% CI for duration of response	1.3, 14.7	1.5, 13.7	2.7, 12.5	1.5, 7.4	1.8, 14.3	1.4, 12.9

Table 18. Response rate and duration of response - FDA derived data sets H0648g

Parameter	ACH n, (%) n = 143	AC n, (%) n = 138	TH n, (%) n = 92	T n, (%) n = 96	H + Chemo n, (%) n = 235	Chemo alone n, (%) n = 234
Confirmed responses (REC); CR+PR [95% CI]	71 (50%) [41,57]	53 (38%) [30,46]	35 (38%) [26,46]	14 (15%) [7,22]	106 (45%) [37,50]	67 (29%) [23,34]
p value X^2 test	0.10		0.001		<0.001	
Confirmed CR (REC)	7 (5%)	4 (3%)	4 (4%)	2 (2%)	11 (5%)	6 (3%)
Median Duration of response for confirmed responses (mos)	9.1	6.4	8.3	4.3	9.1	5.8
25%, 75 % quantile for median duration of response	5.8, 14.9	4.5, 8.5	4.9, 11.0	3.7, 7.4	5.5, 14.9	3.9, 8.5

Time to Treatment Failure

Time to treatment failure was defined as the time from the date of enrollment to the date of progressive disease, death, introduction of additional non-protocol defined anti-tumor therapy, or study discontinuation for any other reason.

Table 19. Time to Treatment Failure - Sponsor derived data set H0648g

Parameter	AC + H N = 143	AC N= 138	T+H N = 92	T N = 96	Chemo + H N = 235	Chemo N =234
No. pts with treatment failure	117	127	70	93	187	220
Median TTF (mos)	7.1	5.6	5.3	2.7		
95% CI	6.2,7.8	4.6,5.6	4.1,7.1	2.0,4.3		
p value (log rank)	0.001		< 0.0001			

Survival

The survival analysis was complicated by the fact that patients who had REC defined progressive disease could "cross-over" and receive Herceptin® with or without other anti-tumor therapy in study H0659g. Table 20 and Figures 5,6, and 7 present the survival data to the cutoff date of March 1998. While there appeared to be no difference in the median survival between subgroups (ACH vs AC and TH vs T), the short term one-year survival was longer for those patients who received Herceptin®. In addition, as can be seen in Figures 5, 6, and 7, there was a great deal of censoring of the data after month 12 indicating that the data were not mature at the time of the analysis. Therefore, this is an interim and not a final survival analysis.

Table 20. Survival H0648g

Parameter	AC + H N = 143	AC N= 138	T+H N = 92	T N = 96	Chemo + H N = 235	Chemo N =234
No. deaths	38	50	32	42	70	92
Median survival (mos)	24.8	24.2	19.3	18.3		
95% CI	18.1, n/a[a]	15.7, n/a	14.2, n/a	12.6, n/a		
p value (log rank)	0.09		0.22		0.03	
One-year survival Percent alive	83%	73%	73%	61%	79%	68%
95% CI	77,89	66,82	66,80	51,71	74,84	62,74
p value (z test)	0.04		0.08		<0.01	

a The upper limit of the 95% confidence interval has not been reached at this time.

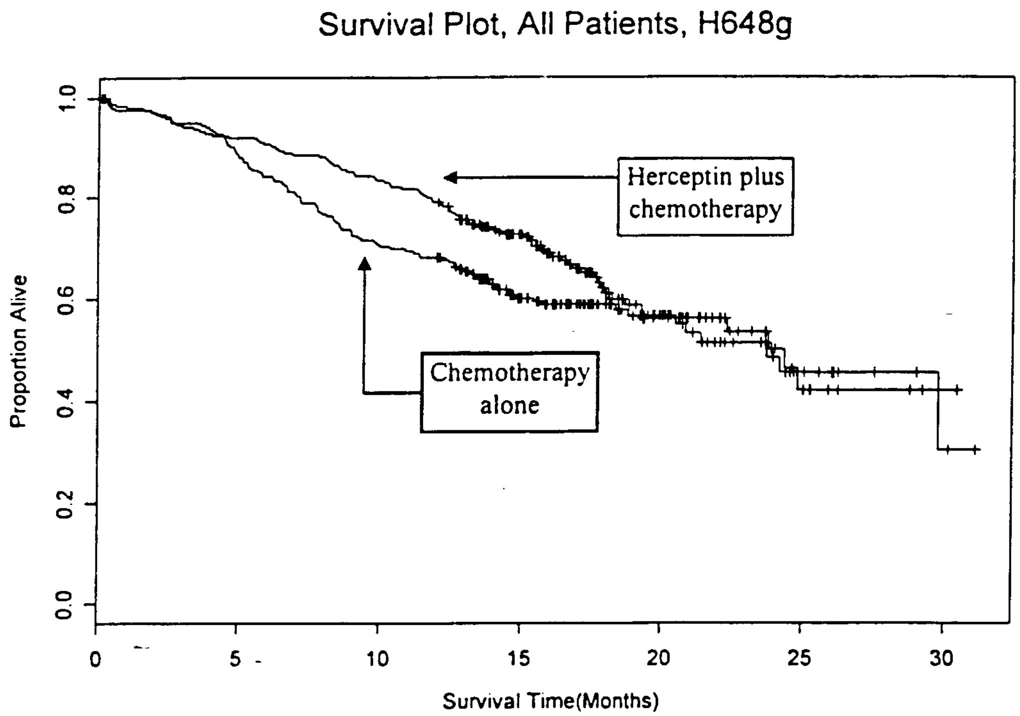

Figure 5. Survival plot for all patients treated on trial H0648g. Comparison of the two randomized arms: Herceptin plus chemotherapy vs. chemotherapy alone.

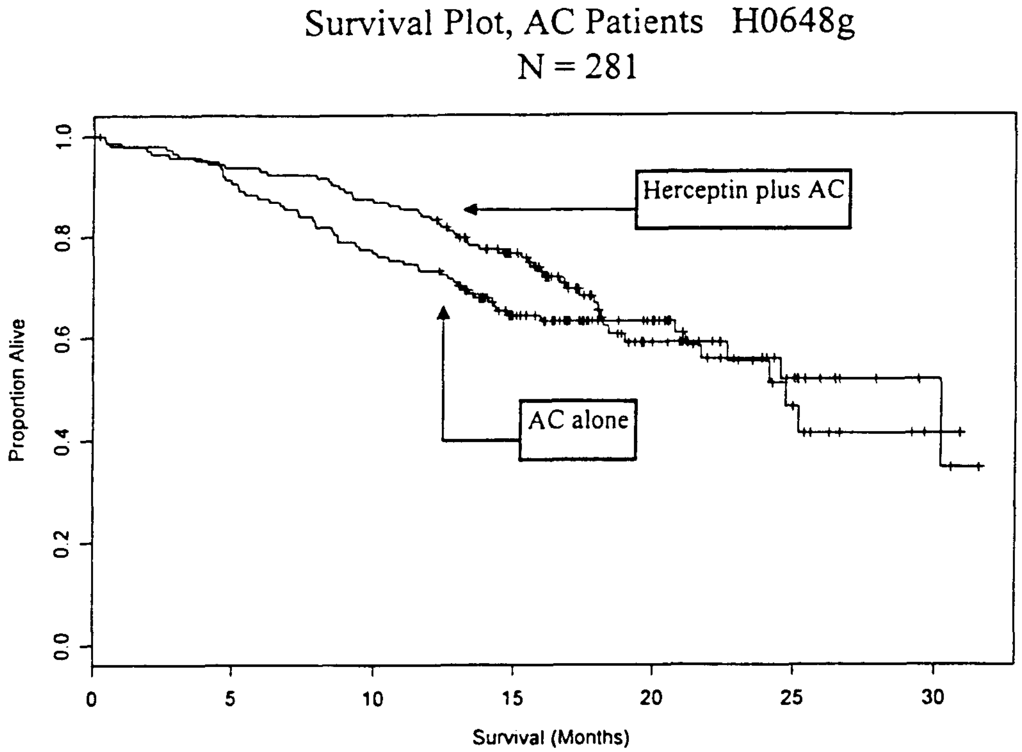

Figure 6. Survival plots for patients treated with AC chemotherapy with and without Herceptin.

Survival Plot, Taxol Patients H0648g
N = 188

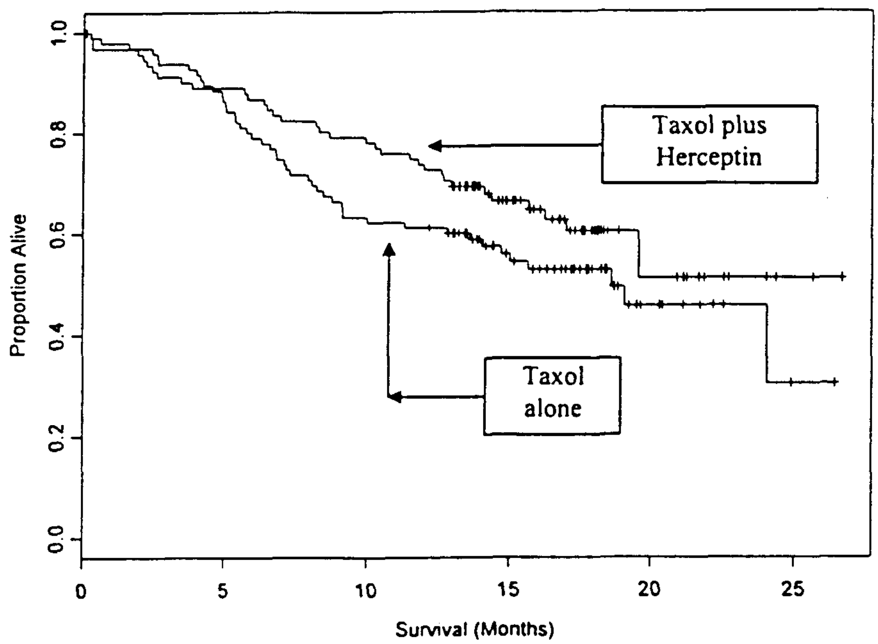

Figure 7. Survival plots for patients treated with paclitaxel chemotherapy with or without Herceptin.

6.6.10 Immunohistochemistry (IHC) testing for protein overexpression of HER2

As a selection criterion for patients enrolled on H0648g, the presence of HER2 protein overexpression in tumor biopsies based upon IHC reading scores of 2+ or 3+ (on a 0 - 3+ scale) was required. It is important to note that when reading the IHC slides the pathologists had been readily able to identify 3+ and 0 scored tumors, whereas, 1+ and 2+ tumors were more difficult to distinguish one from the other. Also, 3+ and 0 scores will outnumber 1+ and 2+ scores on any random sampling. Table 21 lists the number of patients who had 2+ or 3+ protein overexpression. Table 22 shows the response rates (sponsor's data set) for the 2+ and 3+ patients separately. A larger percent of 3+ patients in the treatment arm (H + chemo) responded to therapy compared to 2+ patients; yet, in the control arm (chemo alone), the trend is reversed, with a greater number of 2+ patients responding.

Please see Section 7.0 of this review for a detailed analysis of the level of HER2 overexpression and clinical outcome of patients treated with Herceptin®

Table 21. Responding patients relative to immunohistochemistry score of protein over-expression. H0648g

Parameter	ACH	AC	TH	T	H + Chemo	Chemo alone
No. pts 2+	35	42	24	19	59	61
No. pts 3+	108	96	68	77	176	173

Table 22. Proportion of patients who had a tumor response (2+ vs 3+). H0648g

Parameter	ACH	AC	TH	T	H + Chemo	Chemo alone
2+ patients %	26	38	17	11	22	30
3+ patients %	39	34	24	13	33	25

6.7 Results - Safety H0648g

6.7.1 Adverse events overall

The data set submitted by the sponsor for adverse events was evaluated for selected adverse events which were frequent or by their nature of concern even if not frequent in incidence. In reviewing the case report forms, it was noted that there was considerable variability in the reporting of adverse events between sites with some sites reporting none or almost no adverse events and others reporting many pages worth of adverse events for patients receiving the same therapies; there did tend to be within site consistency in the frequency of reporting which suggests that the differences were not differences in the number of actual events, but differences in methodology between investigators. The data for adverse events appear in Tables 23 and 24 and include percentage of patients enrolled in each subgroup and the number of events which were moderate or severe.

Table 23. Adverse events H0648g. Listing as percent of those enrolled and treated on study H0648g who experienced the event.

Event Term	ACH Percent of patients N = 143	AC Percent of patients N = 135	TH Percent of patients N = 91	T Percent of patients N = 95	H + Chemo Percent of patients N = 234	Chemo alone Percent of patients N = 230
Pain	57	42	61	62	58	51
Back pain	27	15	34	30	31	21
Abdominal pain	23	18	34	22	27	20
Chest pain	20	20	29	28	24	24
Bone pain	7	7	24	18	14	11
Neck pain	10	8	9	5	10	7
Rash	27	17	38	18	31	18
Skin ulcer	6	4	3	1	5	3
Skin discolor.	5	2	2	1	4	2
Rhinitis	22	15	22	5	22	11
Allergic reaction	4	2	8	2	6	2
Anaphylactoid reaction	0	0	1	0	0.4	0
Leukopenia	52	34	24	17	41	26
Anemia	36	26	14	9	27	18
Thrombocytopenia	11	9	3	3	8	6
Hypochromic anemia	6	1	2	2	4	1
Nausea	76	77	51	49	66	66
Fever	56	34	49	23	53	30
Vomiting	53	49	37	28	47	41
Headache	44	31	36	28	41	30
Chills	35	11	41	4	37	8
Insomnia	29	15	25	5	28	14
Arthralgia	8	9	37	21	20	14
Depression	20	12	12	13	17	12
Nausea and vomiting	18	9	14	11	17	10
Flu syndrome	12	6	12	5	12	6
Chills and fever	2	1	5	4	3	2
Cough increased	43	29	41	22	43	25
Dyspnea	42	25	27	26	36	25
Lung disorder	8	3	8	0	8	2
Pleural effusion	6	2	7	5	6	3
Asthma	4	4	5	2	5	3

Table 23 (continued). Adverse events listed as percent of patients with the event

Event Term	ACH	AC	TH	T	H + Chemo	Chemo alone
Peripheral edema	20	17	22	20	21	18
Edema	11	5	10	8	11	6
Tachycardia	10	5	12	4	11	5
CHF	12	1	2	1	8	1
Left heart failure	10	5	5	0	8	3
Cardiomyopathy	7	1	1	0	5	1
Hypotension	7	4	2	3	5	3
Palpitation	6	4	4	2	5	3
Hypertension	3	3	5	4	4	3
Cardiovascular disorder	2	5	3	1	3	3
Stomatitis	30	32	10	7	22	21
Mucous membrane d/o	22	18	11	7	18	14
Mouth ulceration	12	14	4	1	9	9
Taste perversion	11	13	5	3	9	9
Conjunctivitis	8	7	7	2	8	5
Dry mouth	6	9	8	5	7	7
Esophagitis	1	6	0	2	1	4
Epistaxis	7	6	17	4	11	5
Ecchymosis	6	2	8	2	7	2
Rectal hemorrhage	4	1	4	1	4	1
DVT	3	1	1	1	2	1
Hemorrhage	1	1	3	0	2	0.4
Thrombophlebitis	1	1	0	0	1	1
Diarrhea	45	26	45	29	45	26
Dyspepsia	22	20	17	16	20	18
Rectal disorder	7	6	7	0	7	3
Infection	47	31	47	27	46	30
Pharyngitis	30	18	22	14	27	16
Sinusitis	13	6	21	7	16	6
UTI	13	7	18	14	15	9
Herpes simplex	7	9	12	3	9	6
Sepsis	7	7	4	1	6	4
Pneumonia	6	3	2	2	5	3
Herpes zoster	3	3	4	2	3	3
Dehydration	10	4	9	9	10	6
Hypokalemia	13	4	2	3	9	4
Paresthesia	17	11	48	39	29	22
Peripheral neuritis	2	2	23	16	10	8
Neuropathy	3	4	13	5	7	5

Table 24. Adverse events H0648g. Listing by the number of moderate and severe events H0648g

Event Term	ACH Number of moderate and severe events	AC Number of moderate and severe events	TH Number of moderate and severe events	T Number of moderate and severe events	H + Chemo Number of moderate and severe events	Chemo alone Number of moderate and severe events
Pain	81	64	81	79	162	143
Back pain	49	21	43	30	92	51
Abdominal pain	27	14	24	13	51	27
Chest pain	19	29	25	23	44	52
Bone pain	23	8	31	37	54	45
Neck pain	10	7	8	3	18	10
Rash	9	4	16	8	25	12
Skin ulcer	2	5	1	1	3	6
Skin discoloration	0	1	0	0	0	1
Rhinitis	11	2	8	1	19	3
Allergic reaction	1	1	7	2	8	3
Leukopenia	115	93	47	47	162	140
Anemia	73	49	17	10	90	59
Thrombocytopenia	19	18	6	6	25	24
Hypochromic anemia	11	1	4	2	15	3
Nausea	103	105	38	26	141	131
Fever	77	42	36	13	113	55
Vomiting	62	78	34	27	96	105
Headache	41	30	29	15	70	45
Chills	18	10	20	1	38	11
Insomnia	27	18	18	9	45	27
Arthralgia	5	13	60	15	65	28
Depression	31	13	11	12	42	25
Nausea and vomiting	22	7	14	5	36	12
Flu syndrome	14	2	10	0	24	2
Chills and fever	1	1	3	2	4	3
Cough increased	22	22	20	4	42	26
Dyspnea	55	18	24	19	79	37
Lung disorder	4	1	0	3	4	4
Pleural effusion	12	8	9	6	21	14
Asthma	0	2	3	1	3	3

Table 24 (continued). Adverse events listed as number of moderate and severe events.

Event Term	ACH	AC	TH	T	H + Chemo	Chemo alone
Peripheral edema	22	13	27	11	49	24
Edema	6	2	9	6	15	8
Tachycardia	5	3	0	0	5	3
CHF	30	4	7	1	37	5
Left heart failure	17	10	4	0	21	10
Cardiomyopathy	15	1	0	0	15	1
Hypotension	6	1	2	2	8	3
Palpitation	2	13	2	1	4	14
Hypertension	4	6	4	1	8	7
Cardiovascular disorder	0	1	1	0	1	1
Stomatitis	19	27	2	5	21	32
Mucous membrane d/o	25	13	15	4	40	17
Mouth ulceration	4	8	0	0	4	8
Taste perversion	9	6	0	0	9	6
Conjunctivitis	2	7	1	1	3	8
Dry mouth	2	5	9	2	11	7
Esophagitis	0	9	0	2	0	11
Epistaxis	2	1	1	3	3	4
Ecchymosis	2	1	1	1	3	2
Rectal hemorrhage	3	0	0	0	3	0
DVT	4	4	1	1	5	5
Hemorrhage	0	0	0	0	0	0
Thrombophlebitis	2	0	0	0	2	0
Diarrhea	36	18	27	16	63	34
Dyspepsia	22	18	7	4	29	22
Rectal disorder	6	7	3	0	9	7
Infection	47	23	35	25	82	48
Pharyngitis	13	8	3	2	16	10
Sinusitis	8	3	15	5	23	8
UTI	16	10	14	4	30	14
Herpes simplex	9	7	3	1	12	8
Sepsis	14	10	1	1	15	11
Pneumonia	8	3	0	2	8	5
Herpes zoster	3	6	3	1	6	7
Dehydration	13	6	8	7	21	13
Hypokalemia	14	8	1	1	15	9
Paresthesia	3	6	55	22	58	28
Peripheral neuritis	0	2	40	11	40	13
Neuropathy	4	1	16	5	20	6

6.7.2 Adverse events, selected

Due to the problems inherent with the use of preferred terms in which double counting for some entries (e.g. leukopenia and neutropenia) may or may not occur and the fact that the non-scheduled laboratories were not included in the SAS data sets, the FDA did a review of the CRFs for particular adverse events which were of concern and which in the preliminary analysis appeared to be different in incidence between the groups. The adverse events evaluated in this fashion include the following:

1) febrile neutropenia, neutropenic sepsis, neutropenia and fever
2) leukopenia related events which includes the sum of events of leukopenia (if not listed already as neutropenia on the same day) + events of neutropenia + use of G-CSF or GM-CSF + febrile neutropenia episodes
3) anemia related events which includes the sum of events of anemia + erythropoietin use + PRBC transfusions

Table 25 summarizes the data from the CRF review.

Table 25. Selected toxicity events - FDA derived data set H0648g

Parameter	ACH	AC	TH	T	H + Chemo	Chemo alone
Febrile neutropenia, #pts (%)	36 (25)	23 (17)	6 (7)	2 (2)	42 (18)	25 (11)
Febrile neutropenia, # events	45	31	7	2	52	33
Leukopenia related events, #pts (%)	96 (67)	63 (46)	29 (32)	23 (24)	125 (53)	86 (37)
Leukopenia related events, #events	328	244	96	75	424	319
Anemia related events, #pts (%)	55 (38)	37 (27)	16 (17)	11 (11)	71 (30)	48 (21)
Anemia related events, #events	225	91	48	25	273	116
Platelet related events, #pts (%)	18 (13)	13 (9)	6 (7)	4 (4)	24 (10)	17 (7)
Platelet related events, #events	32	30	17	9	49	39
Sepsis, #pts (%)	13 (9)	9 (7)	4 (4)	2 (2)	17 (7)	11 (5)
Sepsis, # events	15	9	4	2	19	11
Hospitalization #pts (%)	69 (48)	53 (38)	23 (25)	35 (36)	92 (40)	88 (38)
Hospitalization #events	115	88	38	58	153	146

6.7.3 Cardiotoxicity

The following sources were used to evaluate cardiotoxicity: CRFs, patient narratives, SAS data sets, and line listings. It was discovered that a substantial amount of data was missing and additional materials were requested and received from the sponsor: CRFs from the Cardiac Response Evaluation Committee (CREC), corrected SAS data sets.

The CREC charter stipulated that the committee evaluate the cardiac events for patients and classify the severity of heart failure using the New York Heart Association Classification system; the charter noted that this was to be performed for the patient's signs and symptoms at presentation and after the institution of therapy for the cardiac disease. The FDA analysis was a review of those same patients using the same criteria, but the severity of cardiac dysfunction was assessed at its nadir or worst status (which was not necessarily the initial presentation or the final outcome). A few patients reviewed by the FDA and the sponsor were not reviewed by the CREC at the time of data submission. Due to the lack of baseline data for cardiac ejection fractions and cardiac assessments for many patients, it was difficult to make comparisons to patients who did not experience cardiac events.

The CREC consisted of two oncologists and one cardiologist all from Memorial Sloan Kettering Cancer Center. They used the following criteria to evaluate patients:

- Cardiomyopathy characterized by a fall in cardiac EF that was either global or more severe in the septum
- Symptoms of CHF
- Associated signs of CHF including but not limited to S3 gallop, tachycardia
- A decline in cardiac EF of at least 5 points to below 55% with signs and symptoms or a fall in cardiac EF of at least 10 points to below 55% without signs and symptoms.

Patients were scored using the NYHA classification system:

- I no limitations
- II comfortable at rest but ordinary activity leads to symptoms
- III comfortable at rest but less than ordinary activity leads to symptoms
- IV symptoms at rest , very limited in activity

The FDA analysis used these same criteria but applied them to the patient's worst status.

The FDA also analyzed the data using the NCI common toxicity criteria which employs the use of Ejection Fraction results, independent of symptoms. The analysis was not appreciably different from that outlined above; primarily it resulted in more grade 2 toxicity and less grade 1 toxicity.

Table 26 provides a profile composite of all patients who experienced cardiotoxicity. Parameters examined include age, prior radiation to the chest, past medical history and risk factors for cardiac disease, cumulative doxorubicin dose, and ejection fraction (EF) at nadir. For those patients who received epirubicin, the cumulative dose was multiplied by

0.5 to normalize it for risk of cardiac toxicity; Henderson et al.[1] have estimated that the risk for cardiotoxicity with epirubicin is approximately half that of doxorubicin on a mg per mg basis. It is important to note that there was only one patient with a cardiac failure event in the paclitaxel alone arm; this patient in fact was diagnosed with *S. aureus* endocarditis and following antibiotic therapy maintained good left ventricular function as evaluated by two follow up echocardiograms. Examination of only those patients with cardiotoxicity revealed that there was little or no difference between the Herceptin® plus chemo arm and chemo alone in terms of baseline factors including age, prior XRT, PMH, cumulative anthracycline dose and baseline EF when examined as a percentage of those with toxicity. There was a trend toward a lower nadir EF in the ACH group compared to the AC alone arm; however, this group had a slightly lower baseline EF at baseline as well, so this trend may not be clinically relevant. The most notable differences were the incidence and severity of cardiac dysfunction. Comparison of the two arms demonstrated that 51 of 235 patients (22%) experienced cardiotoxicity on the Herceptin® plus chemo arm while 11 of 234 (5%) experienced cardiotoxicity on the chemo alone arm. Examination of the AC subgroups reveals that 41 of 144 patients (28%) experienced cardiotoxicity while 10 of 138 AC patients (7%) experienced cardiotoxicity. [Note: one patient in the TH arm received only one dose of paclitaxel due to anaphylaxis; she then went on to received 5 cycles of AC; for the cardiotoxicity analysis only, this patient was included in the ACH arm and this is why the n value is 144 and not 143.] Examination of the severity of cardiac dysfunction reveals that there was a shift toward more NYHA class IV events in the ACH arm compared to the AC arm. In addition, the distribution of events in the TH arm was similar to the AC arm. Finally, the T arm had only one event which, one might argue, was due to co-existing endocarditis and not related to therapy for breast cancer. The data for incidence and severity are depicted graphically in Figure 8. In this figure, class III and IV events are separated from class I and II events in order to display the data in a clinically relevant fashion.

Comparison was also made between cumulative anthracycline doses of the patients who developed cardiotoxicity compared to those patients who did not develop cardiotoxicity. However, the data for the patients who did not develop cardiotoxicity was not collected in as complete a fashion as for those with cardiotoxicity; there was no cumulative anthracycline dose data for 25 patients. A similar comparison of prior chest irradiation and cardiac related PMH was not conducted since the data were even less consistently collected and it was felt that such an analysis would not be possible due to missing data.

The cause of death in patients who developed cardiotoxicity was assessed. Cardiotoxicity was a factor in the deaths of two patients in the ACH subgroup and two patients in the AC subgroup. The two ACH patients and one AC patient also had breast cancer as an associated cause of death; one AC patient clearly died due to cardiac disease. The other AC patient died after receiving Herceptin® in the extension study H0659g.

[1] Henderson IC. Chemotherapy for metastatic disease. In *Breast Diseases*, Second Edition. Harris JR, Hellman S, Henderson IC, Kinne DW (eds). J.B. Lippincott, Philadelphia, 1991, pp 615-7.

Table 26. Profile/demographics of patients who developed cardiotoxicity H0648g. Percent of patients affected per subgroup appear in parentheses ( ).

Parameter	ACH N = 144	AC N = 138	TH N = 91	T N = 96	Herceptin + Chemo	Chemo
# pts with cardiotoxicity	41 (28)	10 (7)	10 (11)	1 (1)	51 (22)	11 (5)
# pts with NYHA class I CHF	10 (7)	6 (4)	5 (5)	0 (0)	15 (6)	6 (3)
# pts with NYHA class II CHF	3 (2)	0 (0)	1 (1)	0 (0)	4 (2)	0 (0)
# pts with NYHA class III CHF	7 (5)	1 (1)	3 (3)	0 (0)	10 (4)	1 (0.4)
# pts with NYHA class IV CHF	21 (15)	3 (2)	1 (1)	1 (1)	22 (9)	4 (2)
# pts with prior XRT	17 (12)	4 (3)	6 (7)	0	23 (10)	4 (2)
# pts with + PMH	23 (16)	6 (4)	7 (8)	1 (1)	30 (13)	7 (3)
XRT + PMH	11 (8)	2 (1)	3 (3)	0	14 (6)	2 (1)
Age, mean	55	50	54	45	55	49
Age, range	26 - 73	37 - 62	36 - 72	45	26 - 73	37 - 62
Nadir EF, median [a]	30	40	42	71	30	44
Nadir EF, CI[a]	10 - 50	15 - 48	20 - 52	71	10 - 52	15 - 71
Baseline EF, median [b]	60	66	62	not available	60	66
Baseline EF, CI [b]	33 - 80	62 - 75	45 - 66		33 - 80	62 - 75
Patients with cardiotoxicity: Cumulative anthracycline dose, median (CI)	350 (108, 578)	358 (290, 482)	281 (60, 432)	222 (222, 222)	348 (74, 552)	351 (222, 482)
Patients without cardiotoxicity: Cumulative anthracycline dose, median[c] (CI)	298 (51, 625)	343 (61, 488)	445 (74, 724)	400 (125, 660)	348 (59, 678)	356 (79, 605)

a Not all patients had post-baseline ejection fractions performed. The n value for each group is as follows: ACH = 40, AC = 10, TH = 9, T = 1.

b Not all patients had baseline ejection fractions performed. The n value for each group is as follows: ACH = 26, AC = 5, TH = 5, T = 0.

c This value includes data from 382 patients. There was no data for cumulative anthracycline dose in 25 patients.

Figure 8 below provides a graphical representation of the incidence and severity of cardiotoxicity. For purposes of comparison, the results from H0649g have been included; however, it must be noted that the patients enrolled on the single agent study H0649g were heavily pre-treated patients with a poorer prognosis at study entry. For details on the H0649g patients, please refer to the safety section for H0649g of this review.

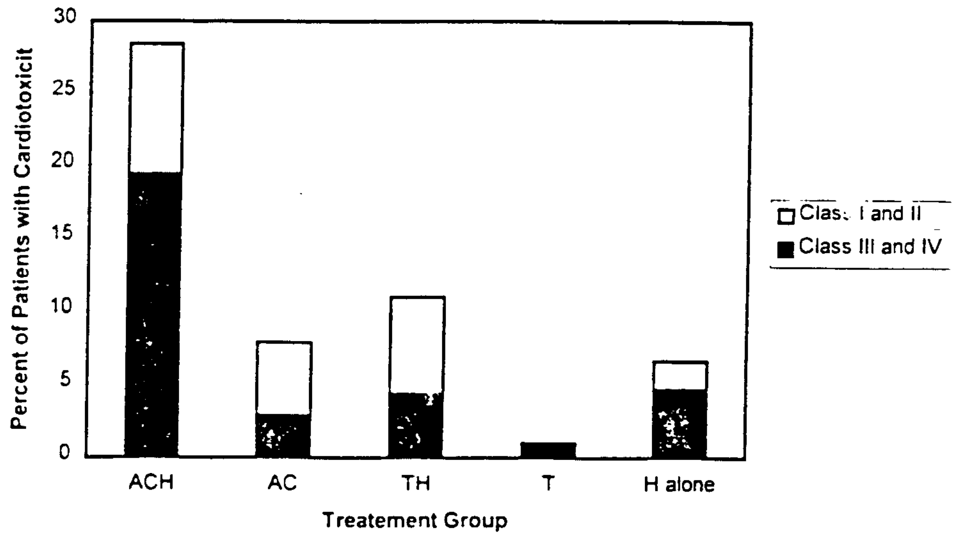

Figure 8. Summary of cardiac events for each treatment subgroup of H0648g and for patients treated with single agent Herceptin® in H0649g. The top of each column represents the total number of events and the shaded and unshaded areas are added to achieve the total. For example, for the ACH arm the percent of class I and II events is 9% and that of class III and IV events is 19.4% and the total is 28.4%. Also, please note that the TH, T and H alone groups had received anthracyclines in the past with the notable exception of two patients in the H alone group and these two had evidence for pre-existing cardiac disease; they did not receive anthracyclines concurrently with Herceptin® as did group ACH.

The FDA also examined the incidence of cardiotoxicity relative to cumulative anthracycline dose in the ACH and AC subgroups. Patients were divided into three dose categories: < 300 mg/m^2, 301 - 450 mg/m^2, and > 450 mg/m^2. It was felt that this permitted examination of therapeutically relevant subgroups; the majority of patients were expected to fall in the 301 - 450 mg/m^2 dose range and in clinical practice this is the range within which most physicians treat their patients. This analysis was based upon the 281 patients who received AC therapy on study with or without Herceptin®. The results are presented in Tables 27 and 28. There is a 6.5 fold and 6.7 fold difference in cardiotoxicity in the < 300 and 301- 450 mg/m^2 dose ranges respectfully and 0.9 fold differences at the higher dose levels. Analysis of class III and IV events reveals even greater differences on the magnitude of 8.3 fold and greater.

Table 27. Incidence of cardiotoxicity (class I - IV) by cumulative dose range H0648g

Dose range	ACH	AC	Multiple Difference or ratio = ACH÷AC
< 300 mg/m2	13%	2%	6.5
301 - 450 mg/m2	40%	6%	6.7
> 450 mg/m2	36%	40%	0.9

Table 28. Incidence of cardiotoxicity (class III - IV) by cumulative dose range H0648g

Dose range	ACH	AC	Multiple Difference or ratio = ACH÷AC
< 300 mg/m2	12%	0%	cannot calculate a defined number
301 - 450 mg/m2	25%	3%	8.3
> 450 mg/m2	27%	20%	1.4

The difference in incidence relative to cumulative dose was also born out in the Kaplan-Meier plots of cumulative anthracycline dose vs. proportion of patients with cardiac dysfunction. See Figures 9 and 10.

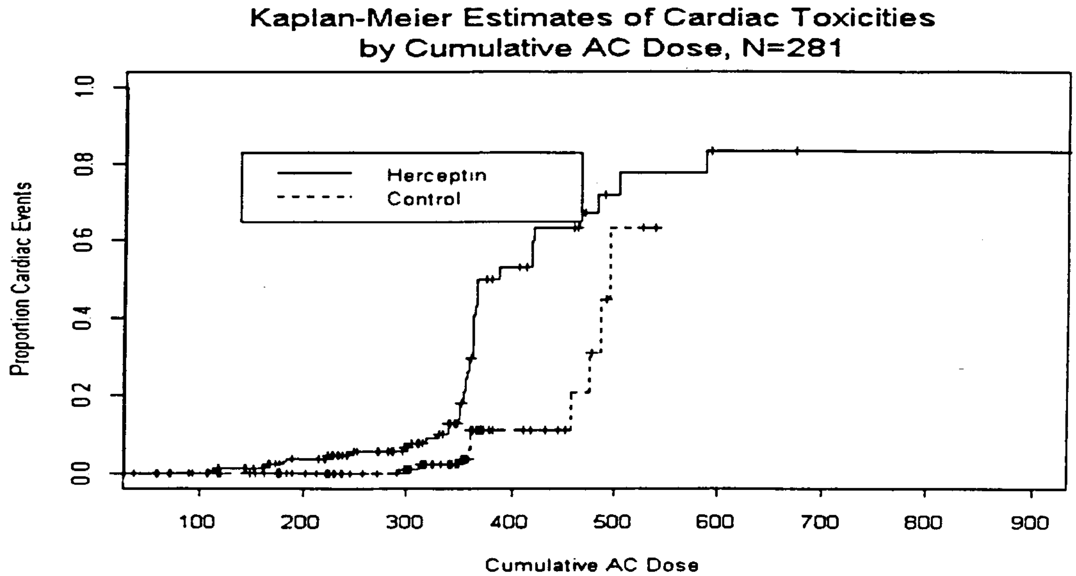

Figure 9. Plot of the proportion of patients (based upon all patients enrolled) who developed cardiotoxicity (NYHA clâsses I - IV) relative to cumulative anthracycline dose. Epirubicin doses were converted to doxorubicin equivalent cardiotoxic doses. This is a comparison of the two AC subgroups: Herceptin® plus AC (Herceptin) vs. AC alone (control).

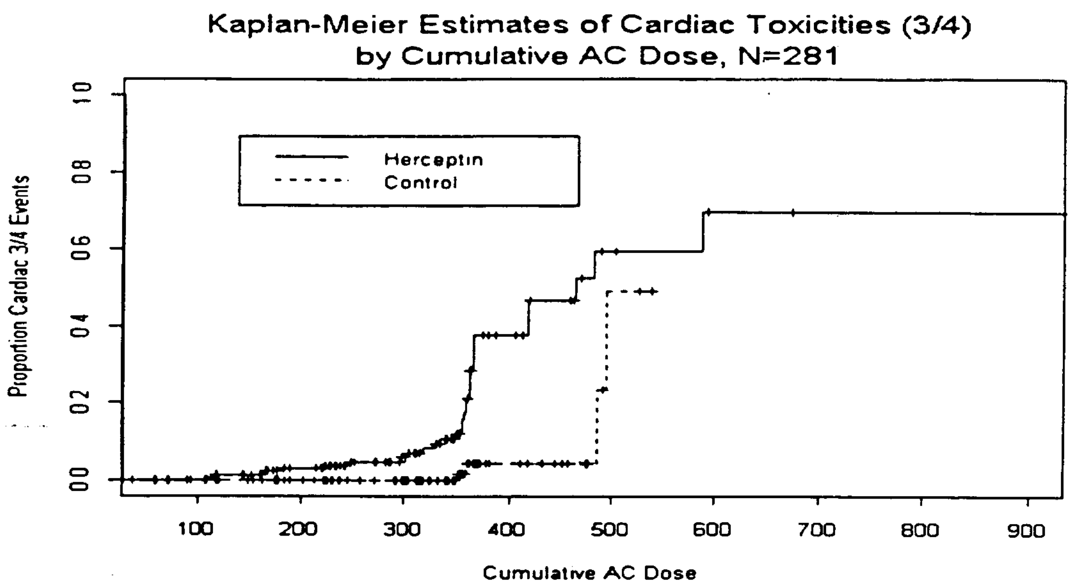

Figure 10. Plot of the proportion of patients (based upon all patients enrolled) who developed cardiotoxicity (NYHA classes III and IV) relative to cumulative anthracycline dose. Epirubicin doses were converted to equivalent cardiotoxic doses. This is a comparison of the two AC subgroups: Herceptin® plus AC (Herceptin) vs. AC alone (control).

The use of dexrazoxane (Zinecard®), an approved cardio-protectant, was examined. The clinical protocol was silent on the use of this agent, neither suggesting nor prohibiting its use; investigators were free to use their own judgement. There were 11 patients in the ACH subgroup and 2 patients in the AC subgroup who received dexrazoxane treatment. The number of doses administered ranged between 1 and 6 with a median of 2 doses. Three of the patients treated with the cardio-protectant developed cardiotoxicity: one class I, one class II and one class III heart failure. All three patients were in the ACH subgroup. Thus, 27% of the ACH patients treated with dexrazoxane developed cardiotoxicity; this rate is comparable to the overall rate for the subgroup of 28%. Neither of the two AC patients developed cardiotoxicity.

Brief summaries of all patients experiencing class III and IV cardiac events and symptoms can be found in Appendix A. These summaries are informative because they provide information on the nature of the events (e.g. orthopnea, ventricular arrhythmias, mural thrombus with subsequent embolic middle cerebral artery stroke), the medications used to treat the patients (e.g. digoxin, ACE inhibitors, beta blockers, diuretics and intravenous dopamine and dobutamine) and details of the ejection fractions (EF) data for each patient. It is important to note that three of the 4 patients on the control arm (AC) subsequently enrolled in the extension study and received Herceptin®; the symptoms of congestive heart failure (CHF) for these three patients worsened during the period of time they were on Herceptin® therapy in the extension study.

7.0 Relationship Between Level of HER2/neu Protein Overexpression and Clinical Benefit

Level of HER2/neu protein overexpression

As discussed earlier in the document, the tumor samples of patients with metastatic breast cancer were tested for the presence of HER2/neu protein over-expression by immunohistochemistry (IHC). Two different antibodies were employed.

4D5 antibody - binds to the extracellular domain of HER2/neu and is the parent antibody of Herceptin

CB11 antibody - binds to the intracellular domain of HER2/neu

Parenthetically, the PMA for the test kit, HercepTest, with the proposed indication for the selection of patients appropriate for treatment with Herceptin® was simultaneously under review by the FDA. The test kit used a polyclonal antibody which binds to the intracellular domain. For all the above assays, tumor samples were scored as 0, 1+, 2+ or 3+. Only patients with scores of 2+ or 3+ were enrolled into studies H0649g (Phase 2) and H0648g (Phase 3).

We examined the data (FDA data sets) for differences in the efficacy endpoints (time to progression and response rate) between patients who were 2+ vs. 3+ overexpressers of the HER2/neu protein by IHC testing. There was one patient scored as 1+ by the protein assay and 2+ by FISH who was entered on study as an exemption; she did not have a tumor response and progressed by week 8. For purposes of the analysis she is included as a 2+ overexpresser.

The distribution of patients who were 2+ or 3+ was comparable between the two study arms of H0648g. The data appear below in Table 29.

Table 29. Percent of patients testing 2+ or 3+ by IHC in study H0648g and H0649g.

HER2 score	ACH N = 143 % (n)	AC N = 138 % (n)	TH N = 92 % (n)	T N = 96 % (n)	H+Chemo N = 235 % (n)	Chemo N = 234 % (n)	H (649g) N = 222 % (n)
2+	24% (35)	30% (42)	26% (24)	20% (19)	25% (59)	26% (61)	22% (50)
3+	76% (108)	70% (96)	74% (68)	80% (77)	75% (176)	74% (173)	78% (172)

The data for the overall response rate (responses sustained for at least 4 weeks) by IHC score (2+ vs. 3+) appear in Table 30. The patients with tumors which scored as 3+ achieved a significantly higher tumor response rate when treated with Herceptin® plus AC or paclitaxel compared with those treated with AC or paclitaxel alone: 53% vs 36%

for ACH 3+ vs AC 3+, respectively and 44% vs 14% for TH 3+ vs T 3+, respectively. The benefit for the patients who were 3+, again, was greater for those in the paclitaxel subgroup. Patients with tumors which scored as 2+ treated with the same regimens showed no difference in response rate between those treated with Herceptin plus chemotherapy and those treated with chemotherapy alone: 40% vs 43% for ACH 2+ vs AC 2+, respectively and 21% vs 16% for TH 2+ vs T 2+, respectively.

Table 30. Response rate by IHC score for HER2/neu protein expression H0648g and H0649g. The denominator value (n) appears in Table 29 for each corresponding subgroup and score.

HER2 score of responders	ACH % (N)	AC % (N)	TH % (N)	T % (N)	H+Chemo % (N)	Chemo % (N)	H % (N)
2+ (CR+PR)	40% (14)	43% (18)	21% (5)	16% (3)	32% (19)	34% (21)	4% (2)
3+ (CR+PR)	53% (57)	36% (35)	44% (30)	14% (11)	49% (87)	27% (46)	17% (29)

We also examined the time to progression for 2+ vs 3+ patients and found that there was no difference in time to progression for patients with 2+ tumors ($p > 0.10$), but there was a significant increase in time to progression for those with 3+ tumors ($p < 0.001$). Statistical testing for an interaction (i.e. testing of the difference between the differe⁻ces of the curves) was significant with a p value < 0.05. The curves for time to progression appear in Figures 11 and 12 and the median time to progression values are in Table 31.

Table 31. Median time to progression of 2+ vs 3+ patients enrolled on H0648

HER2 score by IHC	ACH months	AC months	TH months	T months
2+ (CR+PR)	7.6	7.1	4.4	3.2
95% CI	5.3, 10.1	4.8, 9.7	2.2, 6.6	2.0, 5.6
3+ (CR+PR)	7.3	4.9	7.1	2.2
95% CI	7.1, 9.8	4.5, 6.9	6.7, 12.0	1.8, 4.3

These studies (H0649g and H0648g) were not designed to test whether or not there was a relationship between the impact of Herceptin® on time to pr⁻gression, response rate, or survival and the extent of HER2 overexpression (2+ vs. 3+); therefore, this analysis is exploratory in nature. There were far fewer patients with tumors that scored 2+ and this affects the power of such an analysis. Nonetheless, the observed difference in clinical outcome and the interaction of the level of overexpression were significant.

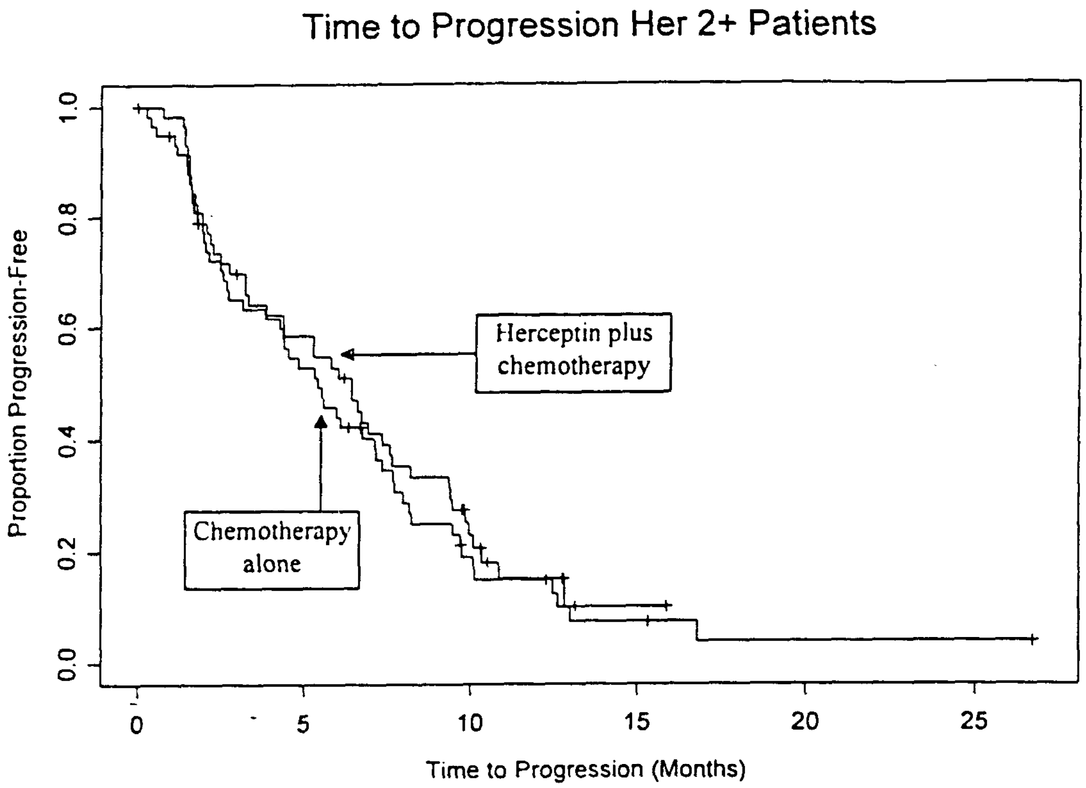

Figure 11. Time to progression for patients whose tumors were scored as 2+ for protein overexpression. Herceptin plus chemotherapy vs chemotherapy alone. Study H0648g

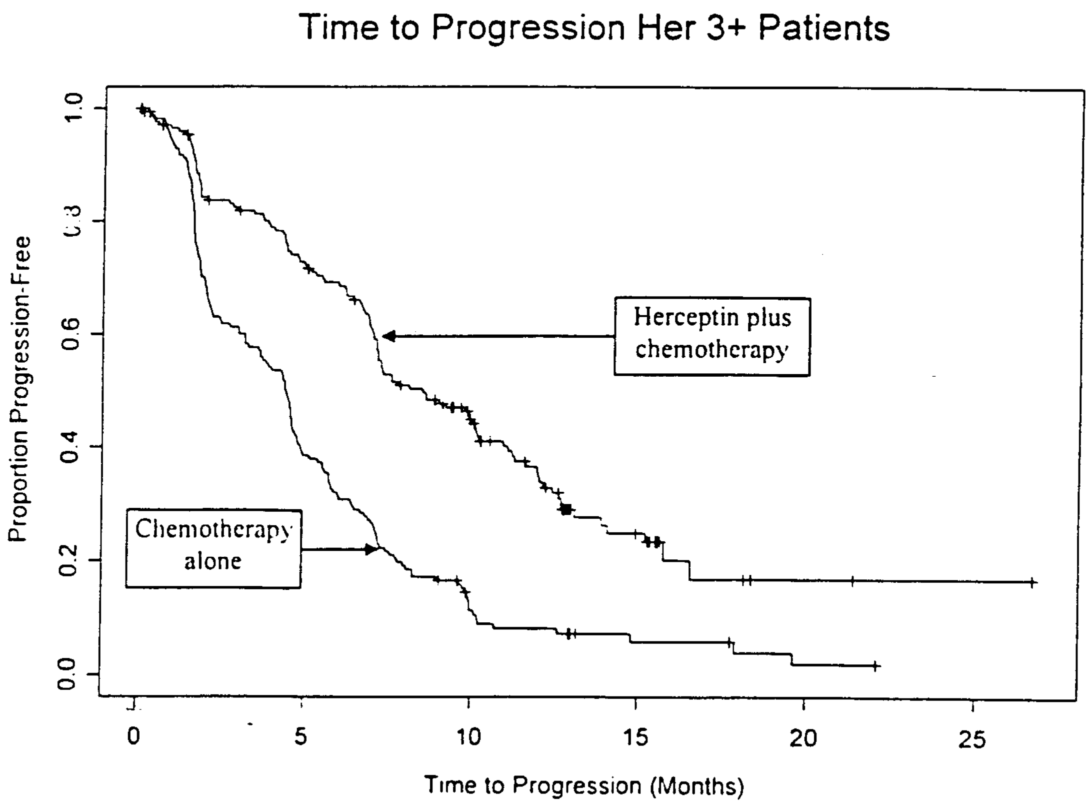

Figure 12. Time to progression for patients whose tumors were scored as 3+ for protein overexpression. Herceptin plus chemotherapy vs chemotherapy alone. Study H0648g.

Conclusion

The impressive relationship between level of overexpression and clinical benefit appears to indicate that there may be a receptor mediated process occurring. One interpretation is that this is indirect proof of concept for the mechanism of action of the product, Herceptin®. Comparison of the response rate data, time to progression data and survival data strongly indicate that patients who benefited from the addition of Herceptin to chemotherapy were those whose tumors were 3+ overexpressers. No benefit was seen for those patients whose tumors were 2+ overexpressers. The question requiring further study in a prospective fashion is whether or not patients whose tumors score as 2+ should have Herceptin® added to chemotherapy and receive the added toxicity without the additional clinical benefit.

8.0 OVERVIEW OF EFFICACY FOR ALL PHASE 2 AND 3 STUDIES

Studies submitted to the BLA:

Study # Phase	Regimen	#pts	Indication	Accrual Status
H0407g Phase 1	Single dose 10, 50, 100, 250, 500 mg	n=16	met. cancer Her2 (1-3+)	closed
H0452g Phase 1	Weekly dosing 10, 50, 100, 250, 500 mg plus MTP	n=17	met. cancer Her2 (1-3+)	closed
H0453g Phase 1	Weekly dosing 10, 50, 100, 250, 500 mg Plus Cisplatin 100 mg/m2 plus MTP	n=15	met. cancer Her2 (1-3+)	closed
H0551g Phase 2	Weekly dosing 250 mg load/100mg weekly plus MTP	n=46	met. breast ca. Her2 (2-3+)	closed
H0552g Phase 2	Weekly dosing 250 mg load/100mg weekly Plus Cisplatin 75 mg/m2 plus MTP	n=39	met. breast ca Her2 (2-3+)	closed
H0648g Phase 3 Pivotal Study	*Weekly dosing 4 mg/kg load 2 mg/kg weekly Plus AC or Paclitaxel vs chemo alone May go to H0659g at PD*	*n=469*	*met. breast ca Her2 (2-3+)*	*closed*
H0649g Phase 2	Weekly dosing 4 mg/kg load 2 mg/kg weekly Plus at PD 2 or 4 mg/kg ± chemo	n=222	met. breast ca Her2 (2-3+)	closed
H0650g Phase 2	Weekly dosing 4 mg/kg load 2 mg/kg weekly or 8 mg/kg load 4 mg/kg weekly	n=62	met. breast ca Her2 (2-3+)	ongoing
H0659g Phase 2	Weekly dosing 4 mg/kg load 2 mg/kg weekly ± antitumor therapy	n=157	met. breast ca Her2 (2-3+)	ongoing
H0693g Phase 2	Weekly dosing 4 mg/kg load 2 mg/kg weekly ± antitumor therapy	n=163	met. breast ca Her2 (any test)	ongoing

Table 32. Efficacy for all Herceptin® studies submitted to the BLA.

Study	REC/INV	CR + PR	MedianTTP (mos)	Median Overall Survival (mos)
H0551g N = 46	INV	5 (11%)	2.8	14
H0649g N = 213	REC	31 (14)	3.1	12.8
H0650g 2mg/kg N =33	INV	7 (21%)	ongoing	ongoing
H0650g 4mg/kg N =29	INV	8 (28%)	ongoing	ongoing
H0693g N = ?	INV	(3%)	n/a	n/a
H0552g N = 46	REC	9 (23%)		11
H0648g ACH N = 143	REC/INV	71 (50%)	7.6	24.8
H0648g AC N = 138	REC/INV	53 (38%)	5.7	24.2
H0648g TH N = 92	REC/INV	35 (38%)	6.7	19.3
H0648g T N = 96	REC/INV	14 (15%)	2.5	18.3
H0659g N = 155	INV	22 (14%)	Ongoing	Ongoing

9.0 REVIEWER'S CONCLUSIONS

9.1 Efficacy of Herceptin®

1. As a single agent, Herceptin® produced sustained objective tumor responses in 14% of patients studied in clinical trial H0649g (N = 222). These patients had all received one or more prior chemotherapies in addition to hormonal therapy for their metastatic disease. Responses were seen in visceral, soft tissue and boney sites of metastasis. Single agent Herceptin® has activity as second or third line therapy for metastatic breast cancer patients who are HER2/neu positive by immunohistochemistry.

2. Patients who were treated with Herceptin® in combination with AC chemotherapy (doxorubicin 60 mg/m^2 or epirubicin 75 mg/m^2 plus cyclophosphamide 600 mg/m^2), experienced a longer time to progression by 2.1 months ($p < 0.001$), a higher response rate (50% vs 38%), and improved short term 1-year survival, but no difference in median overall survival when compared to AC alone (ACH, N = 143; AC, N = 138). These patients had not received chemotherapy for their metastatic disease, though they may have received hormonal therapy. Herceptin® in combination with AC chemotherapy improved time to progression, response rate, and short term survival, but did not improve long term survival when compared to AC alone as first line therapy for patients with metastatic breast cancer whose tumors overexpress HER2/neu by immunohistochemistry (2+, 3+).

3. Patients who were treated with Herceptin® in combination with T chemotherapy (paclitaxel 175 mg/m^2 infused over 3 hours), experienced a longer time to progression by 4.2 months ($p < 0.001$), a higher response rate (35% vs 14%), improved short term 1-year survival, but no difference in median overall survival when compared to T alone (TH, N = 92; T, N = 96). These patients had not received chemotherapy for their metastatic disease, though they may have received hormonal therapy and they did receive prior anthracycline therapy in the adjuvant setting; in addition, a few patients had received prior high dose chemotherapy (BMT or PBSC). Herceptin® in combination with T chemotherapy improved time to progression and short term survival but did not improve long term survival when compared to T alone as first line therapy for patients with metastatic breast cancer whose tumors overexpress HER2/neu by immunohistochemistry (2+, 3+).

4. While neither study H0648g or H0649g was designed to determine a difference in clinical benefit between patients who were 2+ and those who were 3+ by immunohistochemistry testing for HER2/neu protein overexpression, exploratory analyses suggest that 3+ patients benefit more by the addition of Herceptin® to AC or T than do 2+ patients and that when administered as a single agent there is a trend for a higher response rate in 3+ as compared to 2+ patients.

9.2 Safety of Herceptin®

1. Herceptin®, when administered as a single agent, was associated with infusional toxicity commonly seen with other monoclonal antibody therapies: fevers, chills, myalgias, back pain, tumor site pain, nausea, and flu like symptoms. This toxicity appeared to be self-limited and controlled with medications and/or with adjustments to the rate of the infusion. Diarrhea (25%) and abdominal pain (28%) were commonly seen and may be related to the known binding characteristics of parent antibody of Herceptin®, 4D5, to normal gut tissues. Cardiotoxicity (7%) when observed was more often severe in nature and occurred in patients with and without prior anthracycline exposure; although, those without anthracycline exposure did have pre-existing cardiac disease. Anemia (11%) and leukopenia (8%) were noted in this heavily pretreated population. Other adverse events noted were not atypical for the population of patients evaluated. Overall, the toxicity profile when compared to cytotoxic chemotherapies was favorable.

2. Herceptin® in combination with AC therapy (doxorubicin 60 mg/m^2 or epirubicin 75 mg/m^2 plus cyclophosphamide 600 mg/m^2) was associated with the above noted infusional toxicity. Cardiotoxicity was markedly increased with an incidence of 28% overall; of the patients experiencing cardiotoxicity, 68% of the patients suffered events of NYHA class III and IV in severity: this compares with an incidence of 7% in the AC alone patients of whom 40% were class III or IV. Other toxicities which appeared to be increased in incidence and severity when compared to patients receiving AC alone include: anemia, leukopenia, abdominal pain, diarrhea, dyspnea, and infections. Overall, the addition of Herceptin® to AC therapy does result in additional toxicity, primarily infusional toxicity and cardiotoxicity, but also hematologic toxicity, gastrointestinal toxicity, and infectious complications.

3. Herceptin® in combination with paclitaxel (175 mg/m^2 as a 3 hour infusion) was associated with the above noted infusional toxicity. The 11% incidence of cardiotoxicity was markedly increased over those treated with paclitaxel alone (1%). Forty percent of the events were NYHA class III and IV in severity. This compared with an incidence of 1% in the patients treated with T alone. Other toxicities which appeared to be increased when compared to patients receiving paclitaxel alone include: anemia, leukopenia, abdominal pain, diarrhea, vomiting, increased cough, infections, and neurotoxicities. The TH patients received more paclitaxel therapy than did the T patients overall; therefore, it is difficult to estimate how much additional toxicity can be attributed to the addition of Herceptin® for the non-cardiac and non-infusional toxicities. Overall, the addition of Herceptin® to paclitaxel therapy did result in additional toxicity, primarily infusional toxicity, cardiotoxicity, and gastrointestinal toxicity.

4. Clinical studies H0648g and H0649g as well as all other studies conducted with Herceptin® had not been designed to adequately measure the rate of cardiotoxicity,

the risk factors for developing cardiotoxicity, or the mechanism of cardiac damage. There was insufficient information from which to conclude which patients are most at risk, what specific role anthracycline therapy may or may not have in the development of toxicity, and what rate of toxicity would be seen in anthracycline naïve patients without pre-existing cardiac disease. It can only be concluded that: a) Herceptin® was associated with cardiotoxicity when administered alone, b) Herceptin® was associated with an increased incidence of cardiotoxicity when administered in combination with AC or T chemotherapies compared to AC or T alone, and c) Herceptin® administered in patients who recovered from previous cardiotoxicity (either from AC alone, ACH, or pre-existing cardiac disease) was associated with a deterioration in cardiac function in some patients.

9.3 Administration of Herceptin®

1. It cannot be determined from those studies conducted to date, whether or not alternative schedules of Herceptin® have the same level of efficacy as weekly administration until the time of progressive disease. A shorter duration of therapy may or may not be equally efficacious.

2. Pharmacokinetic data from the clinical and pre-clinical studies suggest that when combined with paclitaxel, Herceptin® serum concentrations are increased compared to administration of Herceptin® as a single agent. This same effect was not apparent for the combination with AC therapy.

3. The increased incidence of unexpected toxicities observed when Herceptin® was combined with AC and T suggests that thorough testing of other combinations be conducted prior to more widespread use.

9.4 Risk/Benefit Conclusions

1. The potential benefits of tumor response outweigh the toxicities associated with Herceptin®, when used as a single agent, particularly when compared to alternative available second and third line therapies for metastatic breast cancer.

2. The additional toxicities associated with the addition of Herceptin® to AC chemotherapy outweigh the clinical benefit in time to progression when compared to AC alone. Particularly, the 28% incidence of cardiotoxicity may lead to additional morbidity which overshadows the 2.1 month improvement in time to progression. While the sponsor has noted that some patients may be treated for their cardiotoxicity, it should be made clear that this therapy at times must be quite aggressive (dobutamine and dopamine required in some cases).

3. The potential benefit of improved time to progression with the addition of Herceptin® to T chemotherapy outweighs the toxicities when compared to T alone.

9.5 Other indications

1. No conclusions can be made regarding the use of single agent Herceptin® as first line therapy for metastatic breast cancer.

2. No conclusions can be made regarding the use of Herceptin® in combination with any other agents for the treatment of metastatic breast cancer.

10.0 APPENDIX A

Patient summaries: NYHA Class III and IV Cardiotoxicity

[Format of summaries: (age), (PMH = cardiac risk factors or history of cardiac disorder), (chest XRT), (cumulative anthracycline dose), (number of cycles chemo), (number of doses Herceptin®), (clinical summary), (EF summary)]

10.1 AC + Herceptin®

61 y/o, no PMH, no XRT, 319 mg/m^2; 6 cycles AC, 24 doses Herceptin®; Herceptin® stopped for cardiotoxicity. Pt had DOE, orthopnea, cough with a 64% fall in EF from baseline. She was treated with ACE inhibitors and diuretics. Her symptoms worsened and she was admitted to the hospital. She experienced an episode of ventricular tachycardia while walking on the ward and was begun on amiodarone. A second episode of ventricular tachycardia occurred and she become unresponsive requiring resuscitation. She was ultimately discharged on dig and an ACE inhibitor with no follow up after 9/97. EF values proceed chronologically from a baseline of 56% to 36% to 20%.

56 y/o, +PMH, no XRT, 116 mg/m^2; 2 cycles AC, 27 doses Herceptin®; AC was stopped for cardiotoxicity and Herceptin® was continued. She was admitted with neutropenia and fever during which time she also went into cardiac failure with ARDS; an open lung biopsy demonstrated poorly differentiated granulomas and mild interstitial chronic inflammation with minimal fibrosis. She was ultimately extubated and continued Herceptin® with paclitaxel instead of doxorubicin. Her EF fell 68% from baseline and the last echo showed evidence of possible laminated apical thrombus for which the patient did not receive anticoagulant therapy; the endomyocardial biopsy revealed hypertrophy and sarcoplasmic vacuoles but no inflammation, necrosis or fibrosis. She continued Herceptin® alone then went on vacation. While abroad she experienced a sudden LOC with CT evidence of large left middle cerebral artery stroke with massive brain edema and herniation. She was stabilized, returned to the U.S. and recovered in a nursing home; she was left with the residua of expressive aphasia and a dense right hemiparesis. EF values proceed chronologically from a baseline of 63% to 40% to 37% to 33% to 20%.

33 y/o, no PMH, +XRT, 355 mg/m^2; 6 cycles AC and 32 doses of Herceptin®. She developed symptoms of DOE, 3 pillow orthopnea, PND and was hospitalized with ECG HR of 154bpm, S3, BP = 80/40 mm Hg, 2+ JVD, 2+ bibasilar rales; her symptoms worsened and she was sent to the ICU. She was treated with O2, dig, diuretics, an ACE

inhibitor and coumadin. There was no baseline, but her EFs proceeded as follows: 65% to 10% to 30%.

67 y/o, +PMH, no XRT, 301 mg/m^2; 5 cycles AC and 15 doses Herceptin®. She developed fatigue, increasing dyspnea, orthopnea; she was tachycardic, tachypneic, S3, with clear lungs. CXR Kerley B lines. Herceptin® was discontinued and she was treated with diuretics and dig. Subsequent ECG showed possible evidence of inferior or anterolateral ischemia and by echo the EF fell 54%. An ACE inhibitor was added. EF values proceed chronologically from a baseline of 61% to 28% to 46% to 37%.

58 y/o, + PMH, + XRT, 343 mg/m^2; 6 cycles AC and 27 doses Herceptin®. She presented with SOB and productive cough and was diagnosed with pneumonia; but, a CXR also showed cardiomegaly and new bilateral pleural effusions. She was treated with diuretics. Herceptin® was discontinued and subsequently the patient requested removal from the study. Patient required home oxygen and could not walk farther than the width of her house at her best. EF values proceed chronologically from a baseline of 64% to 54% to 25%.

65 y/o, no PMH, no XRT, 354 mg/m^2; 6 cycles of AC and 27 doses Herceptin®. She presented with increasing SOB, orthopnea, PND and peripheral edema; she had S3, bilateral pleural effusions and was treated with diuretics and an ACE inhibitor, but her symptoms worsened and she was admitted with 4 pillow orthopnea, PND, dyspnea at rest. Dobutamine and diuretics were instituted, and she had to be sent home with IV dobutamine. Herceptin® was discontinued then resumed one month later. She worsened again and was readmitted and treated with milrinone. Herceptin® was discontinued permanently. She was in need of a left mastectomy which could not be performed due to severe cardiac dysfunction. She remained dependent on dobutamine infusions. EF values proceed chronologically from a baseline of 55% to 50% to 25% to 25%.

58 y/o, + PMH, +XRT, 292 mg/m^2; 6 cycles AC and 38 doses Herceptin®. Chemotherapy was stopped for an "asymptomatic" >20% drop in EF but Herceptin® was continued. After a repeat MUGA demonstrating a further decline in EF, the patient did note SOB, orthopnea, PND and chest heaviness increasing over the last few months. She was treated with diuretics and an ACE inhibitor. Her symptoms worsened and Herceptin® was discontinued. She was treated with dig and a beta blocker. EF values proceed chronologically from a baseline of 63% to 44% to 32% to 39% to 24% to 28% to 47%.

38 y/o, no PMH, no XRT, 360 mg/m^2; 6 cycles AC and

one year of weekly Herceptin® (~ 52 doses). The patient experienced an asymptomatic fall in EF which led to the discontinuation of AC but Herceptin® was continued. She presented with marked dyspnea and Herceptin® was discontinued. There is no notation of other treatment. EF values proceed chronologically from a baseline of 67% to 52% to 43% to 35% to 41% to 34% to 70%.

45 y/o, + PMH, no XRT, 350 mg/m^2; 6 cycles AC and 78 doses of Herceptin®. She presented with increasing SOB and orthopnea which led to a hospital admission for respiratory distress and hypertension. She was diagnosed with CHF and treated with O2, nitroglycerin, solumedrol, morphine, diuretic and an ACE inhibitor. She improved and was sent home on medications. Herceptin® was not continued. She was readmitted 4 months later with chest pain, and ruled out for an MI. Herceptin® was continued. EF values proceed chronologically from a baseline of 55% to 50% to 30% to 30% to 35%.

63 y/o, +PMH, no XRT, 347 mg/m^2; 6 cycles of AC and ~27 doses Herceptin®. The patient presented with DOE, orthopnea and was diagnosed with CHF. She was treated with diuretics. Herceptin® was stopped. Losartan was added. A stress test showed an old anterior wall infarct. A Cardiolyte SPECT scan showed EF 39%, frequent PVC's, ventricular tachycardia and effort induced septal and posterior wall ischemia. EF fell further and the patient withdrew her consent from the study due to the fall in EF. There was no baseline but her EF's proceeded as follows: 37% to 35% to 39% to 35% to 30%.

49 y/o, no PMH, no XRT, 583 mg/m^2; 10 cycles AC and ~36 doses Herceptin®. The investigator discussed, with the patient, a tumor board recommendation to switch her therapy to tamoxifen due to a decrease in her EF, but a "second opinion" recommended that she continue AC-Herceptin® therapy. The patient elected to continue AC-Herceptin® and dexrazoxane was added to regimen. The investigator scored this pt as CR for tumor response. The patient presented later with SOB and ankle edema was diagnosed with CHF and treated with ACE inhibitor, dig, nitroglycerin, and a diuretic. Herceptin® was held. The patient was admitted for a DVT and continued on cardiac medications, coumadin, and tamoxifen. Herceptin® was restarted. EF continued to fall and Herceptin® was stopped again. EF improved thereafter. EF values proceed chronologically from a baseline of 70% to 58% to 56% to 53% to 41% to 29% to 26% then up to 50%.

39 y/o, no PMH, no XRT, 350 mg/m^2; 6 cycles of AC and 27 doses Herceptin®. She presented with increasing SOB, leg edema, and hepatomegaly. She was admitted to the hospital, diagnosed with CHF and

treated with diuretics and lorazepam. She was readmitted within 10 days with worsening SOB and a falling EF and was treated with an ACE inhibitor, diuretic, nitrates, dopamine, dobutamine and dig. Herceptin® was stopped. There was no baseline but her EF's proceeded as follows: 30% to 15% to 39%.

72 y/o, no PMH, +XRT, 216 mg/m^2 (Epirubicin); 3 cycles of AC and 9 doses of Herceptin®. The patient presented with SOB, tachycardia, tachypnea, bibasilar rales, labial cyanosis and was diagnosed with CHF; she was treated with diuretics. AC and Herceptin® were stopped. She then enrolled on the extension study H0659g two months later and received vinorelbine and Herceptin®, but only tolerated one dose vinorelbine. EF values proceed chronologically from a baseline of 80% to 39%.

50 y/o, + PMH, +XRT, 480 mg/m^2; 6 cycles AC and 52 doses Herceptin®. She presented with increasing SOB, pleural effusion and was diagnosed with CHF. She was treated with diuretics and dig; Herceptin® was continued. The patient ultimately requested removal from the study. Baseline EF was 68% but no subsequent EF's were reported. Echo demonstrated global hypokinesis. Fractional shortening was reported as proceeding chronologically from 22% to 21% to 11% to 21% (with normal being > 30%).

34 y/o , no PMH, no XRT, 449 mg/m^2 (Epirubicin); 6 cycles AC and 38 doses of Herceptin®. She presented with dyspnea and tachycardia and was hospitalized and diagnosed with CHF. She was treated with diuretics, dig, and cortensolon. Later she developed an S3 and edema. Herceptin® was held and later restarted. CHF continued to worsen over the next 4 months without any additional information. There was no baseline, but her EF's proceeded as follows: 36% to 23% and fractional shortening fell to 15% then up to 26%.

47 y/o, no PMH, no XRT, 345 mg/m^2; 6 cycles AC and 22 doses Herceptin®. The patient presented with increasing SOB, DOE, pedal edema, chest pain; CHF was diagnosed and she was treated with ACE inhibitor, dig, diuretics. Herceptin® was stopped. There was no baseline, but her EF's proceeded as follows: 40% to 25% to 45%.

54 y/o, + PMH, +XRT, 358 mg/m^2; 6 cycles AC and 41 doses Herceptin®. Due to spinal cord compression, the patient spent 80-100% of the day sitting or in bed; she also developed a DVT and lower extremity edema. A MUGA scan revealed a decreased EF and the patient was diagnosed with CHF; she was treated with diuretics and ACE

inhibitor. Herceptin was continued. EF values proceed chronologically from a baseline of 78% to 38% to 32%.

53 y/o, + PMH, no XRT;, 324 mg/m^2; 6 cycles AC and 36 doses Herceptin®. The patient had baseline SOB and pulmonary involvement with tumor. She was hospitalized for SOB and chest pain and noted to have an EF decreased by 31%. She was treated with a diuretic and oxygen. Herceptin® was continued at full dose, AC was continued at a reduced dose, and she received radiation therapy to the L-S spine. EF values proceed chronologically from a baseline of 58% to 40% to 50%.

67 y/o, + PMH, no XRT, 244 mg/m^2; 6 cycles AC and 29 doses Herceptin®. The patient presented with increasing DOE, lower extremity edema and PND. She was treated with an ACE inhibitor and diuretics. Herceptin® was stopped. EF values proceed chronologically from a baseline of 60% to 45% to 26% to 35%.

68 y/o, + PMH, no XRT, 180 mg/m^2; 3 cycles AC and 10 doses Herceptin®. The patient presented with worsening SOB 5 days after a blood transfusion. She was admitted to the hospital, JVP was noted to be elevated and she was diagnosed with CHF. She was treated with diuretics. Herceptin® was stopped. She was readmitted one month later with increasing SOB, orthopnea, pedal edema and was treated with diuretics and an ace inhibitor. She was noted to have a patent foramen ovale on echo. Two months later SOB worsened again and she required an increase in her diuretic dosage. She was admitted to hospice due to SOB and inability to get around and died 1-2 months thereafter. Cause of death is not clear but felt to be due to both CHF and metastatic breast cancer. EF values proceed chronologically from a baseline of 50% to 30% to 11% to 18%.

72 y/o, + PMH, no XRT, 255 mg/m^2; 6 cycles AC and 21 doses Herceptin®. The patient was admitted to the hospital with nausea, vomiting, fever, SOB. Initially she was diagnosed with pneumonia then an echo revealed evidence of cardiomyopathy. She was diagnosed with CHF and treated with dig, diuretics, metolazone, and an ACE inhibitor. Herceptin® was stopped and one month later the patient requested removal from the study. EF values proceed chronologically from a baseline of 52% to 22%.

73 y/o, + PMH, +XRT, 412 mg/m^2; 7 cycles AC and 61 doses Herceptin®. The patient presented with increasing SOB, orthopnea, and PND; her heart rate was 119 bpm and RR was 22/min with bibasilar rales noted. She was hospitalized and diagnosed with CHF and possibly a silent MI based on ECG findings; she was treated with diuretics, dig, and

an ACE inhibitor. The patient was readmitted the day of discharge with SOB and diagnosed with exacerbation of CHF. Herceptin® was initially stopped then restarted. EF values proceed chronologically from a baseline of 33% to 40% to 33%.

69 y/o, +PMH, no XRT, 355 mg/m^2; 6 cycles AC and 40 doses Herceptin®. She was hospitalized for increasing SOB and was diagnosed with CHF. She was treated with diuretics and dig. Herceptin® was continued. She continued to have SOB with mild exertion. She developed CNS metastases and spinal metastases requiring XRT. The patient reportedly died due to progressive disease. EF values proceed chronologically from a baseline of 55% to 58% to 55% to 55% to 35%.

56 y/o, +PMH, +XRT, 402 mg/m^2; 8 cycles AC and 50 doses Herceptin®. During her 3rd hospitalization for infection she was diagnosed with CHF and treated with diuretics. Herceptin® was continued. She was discontinued from the study for progressive disease and at that time she had SOB and a large pleural effusion. Her diuretic dose was increased and she was then enrolled in the extension study H0659g. She was readmitted for worsening CHF and a thoracentesis was performed, but no pathology results had been provided. She died 3 days after her last dose of Herceptin®. There was no baseline but her EF's proceeded as follows: 50% to 45% to 25%.

49 y/o, +PMH, +XRT, 449 mg/m^2; 8 cycles AC and 67 doses Herceptin®. She presented with SOB, orthopnea, PND, chest discomfort, and palpitations. She had a gallop, bibasilar crackles. She was treated with diuretics, dig, quinapril. The ECG demonstrated evidence of an anterior myocardial infarction, tachycardia and a left anterior fasicular block. Echo showed a 50% reduction in EF from 2 months earlier. Herceptin® was continued. She continued to have palpitations, fatigue, SOB and chest pain. SPECT scan of the heart demonstrated a large fixed severe anterior antero-apical defect. A beta-blocker was added. A cardiac catheterization revealed normal coronary arteries with small lumen caliber, a reduced EF and moderate to severe left ventricle systolic dysfunction. There was no additional follow up. There was no baseline, but her EF's proceeded as follows: 40% to 20% to 37% to 30%.

59 y/o, no PMH, + XRT, 345 mg/m^2; 6 cycles AC and 25 doses Herceptin®. She presented with SOB and was ruled out for pulmonary embolism. Her symptoms and signs progressed as dyspnea, orthopnea, with new bilateral pleural effusions, hepatomegaly, increased JVP, bilateral lower extremity edema and sinus tachycardia. She was admitted to the ICU and diagnosed with CHF. She was treated with diuretics, nitroglycerin, and an ACE inhibitor. Herceptin® was stopped.

Approximately 6 months later the patient's symptoms had improved and her EF had increased. There was no baseline but her EF's proceeded as follows: 29% to 35% to 42%.

70 y/o, +PMH, no XRT, 600 mg/m^2 (epirubicin); 8 cycles AC and 31 doses Herceptin®. She presented to clinic after an episode of loss of consciousness following symptoms of palpitations and SOB which prompted the patient to take several medications at the same time: nitroglycerin, a calcium channel blocker, and lorazepam. Her EF on echo was normal, but reduced from baseline. No additional therapy was provided. She continued Herceptin®. Approximately 4 months later a MUGA scan demonstrated a 50% fall in EF. 8 days later she was admitted for SOB, cough, and chest pain with exertion which resolved with nitroglycerin. She also had a new left bundle branch block on ECG. She was treated with diuretics, an ACE inhibitor, a beta blocker and her calcium channel blocker was stopped. Herceptin® was stopped. EF values proceed chronologically from a baseline of 62% to 50% to 58% to 24%.

64 y/o, + PMH, + XRT, 317 mg/m^2; 6 cycles of AC and 36 doses Herceptin®. The patient complained of SOB, extreme fatigue, and inability to perform daily tasks due to fatigue and SOB. No medications were prescribed. There was no baseline, but her EF's proceeded as follows: 61% to 51% to 50%.

10.2 AC Alone

43 y/o, + PMH, no XRT, 348 mg/m^2; 6 cycles of AC. The patient presented with SOB on exertion and fatigue. CHF was diagnosed and she was treated with diuretics and an ACE inhibitor. Synthroid was begun for hypothyroidism and dig, nitrates and quinapril were added. She was enrolled in the extension study H0659g and treated with Herceptin® and tamoxifen. She was admitted to the hospital with increasing SOB, chest pain, and refractory peripheral edema. She also had a bilirubin of 5.6. Herceptin® was stopped and the patient refused to return for further follow up or treatment. There was no baseline, but her EF's proceeded as follows: 15%.

37 y/o , no PMH, + XRT, 480 mgm^2; 8 cycles AC. The patient presented with progressive SOB and pleural effusions; thoracentesis revealed a transudate with no malignant cells. Ultimately, she was admitted with severe dyspnea at rest with RR of 28, S3 and S4, JVD, and tachycardia at 140 bpm. She was treated with dig, diuretics, oxygen, and dobutamine. The patient had ventricular tachycardia and required resuscitation. She had an extremely tenuous course and died 6 days later with cardiac failure and renal failure. EF values proceeded

chronologically from a baseline of 66% to 50% to 53% to 56% to 41% to 35% to 33%.

54 y/o, + PMH, no XRT, 344 mg/m^2; 6 cycles AC.
The patient presented with SOB and chest tightness; she had an S3, RR = 44/min and HR = 133 bpm. She was diagnosed with CHF. Cardiac catheterization showed normal coronaries. She was treated with diuretic, dig and an ACE inhibitor. Her cancer progressed with brain mets and she was treated with radiation. Following this she enrolled in the extension study H0659g and received paclitaxel and Herceptin®. She developed SOB and hypotension while receiving paclitaxel and was admitted for exacerbation of CHF. She was treated with diuretics, dig, and an ACE inhibitor. Her therapy was switched to vinorelbine and Herceptin® and again to 5FU/LCV and Herceptin®. She was admitted with syncope and chest pain. An MI was ruled out, but the patient had increasing respiratory distress. She died 8 days later in the hospital. EF values proceed chronologically from a baseline of 62% to 25% to 47% (then entered H0659g) to 20%.

53 y/o, +PMH, +XRT; 482 mgm^2; 8 cycles of AC. The patient had an asymptomatic low EF at time of progressive disease. She enrolled in extension study H0659g and received Herceptin® alone for 4 or 5 doses. She presented with DOE and fatigue. She was diagnosed with CHF and treated with diuretics and vasodilators. Cardiac biopsy was performed and was negative for myocarditis, doxorubicin cardiotoxicity, and inflammatory myopathy. Herceptin® was stopped. There was some improvement in function and she went on to be treated with tamoxifen, docetaxel and vinorelbine (separately). There was no baseline, but her EF's proceeded as follows: 46% (after AC alone) to <20% (after herceptin).

10.3 Taxol plus Herceptin®

72 y/o, no PMH, +XRT; 60 mg/m^2 prior doxorubicin; 4 cycles of paclitaxel and 26 doses Herceptin®. The patient complained of mild to moderate SOB on the day of Herceptin® infusions and continuing up to 7 days following. She developed profound fatigue and decreased activity level with increased JVP and 1+ lower extremity edema. A 33% fall in her EF was noted and Herceptin® was continued. The patient later requested discontinuation of Herceptin®. After developing progressive disease, she enrolled in the extension study H0659g. There was no baseline, but her EF's proceeded as follows: 60% to 40%.

66 y/o, + PMH, +XRT; 241 mg/m^2 prior doxorubicin; 11 cycles paclitaxel and ~ 30 doses Herceptin®. She was admitted to the hospital with SOB, palpitations, swelling in the foot. An EKG

demonstrated atrial fibrillation and ST depression inferiorly. She was treated with a calcium channel blocker, diuretic, digoxin, heparin and oxygen. She converted to sinus rhythm. Echocardiogram showed concentric LVH, aortic sclerosis, and a PAP = 41. There was no baseline, but her EF's proceeded as follows: 58% to 52%.

54 y/o, no PMH, + XRT; 360 mg/m² prior doxorubicin; 8 cycles paclitaxel and ~ 42 doses Herceptin®. She had baseline pleural effusions shown to be malignant; they were drained by thoracentesis. She complained of worsening DOE, orthopnea, PND and repeat thoracentesis demonstrated a transudate. CXR showed cardiomegaly. She was treated with diuretics, and an ACE inhibitor. Her symptoms improved and she continued on Herceptin®. EF fell from a baseline of 62% to 45%.

36 y/o, +PMH, no XRT; 406 mg/m² prior doxorubicin; 2 cycles paclitaxel and 8 doses Herceptin®. The patient had mediastinal involvement with tumor at baseline. Her first hospitalization was for right flank pain, RUQ pain, weakness, and SOB. She later developed massive lower extremity edema. Her second hospitalization was for nausea and vomiting, severe neuropathy, increased liver function studies, acute renal failure and CHF. She was treated with diuretics and oxygen. A restaging CT scan demonstrated complete opacification of the lung and she was diagnosed by a pulmonologist with respiratory failure due to CHF vs pneumonitis. The patient died the next day. EF at baseline was 45% with no follow up EF's.

10.4 Taxol alone

45 y/o, +PMH, no XRT; 222 mg/m² prior doxorubicin; 2 cycles paclitaxel. The patient was admitted to the hospital with respiratory distress; she had rales bilaterally and an increased JVP with pedal edema. EKG showed atrial fibrillation with a ventricular rate of 123 bpm. Pleural effusion thoracentesis pathology demonstrated malignant cells. She was then diagnosed with *S. aureaus* endocarditis. Echo done at the same time demonstrated a normal EF. She died shortly thereafter due to progressive disease in the lung. There was no baseline, but her EF's proceeded as follows: 71% to 72%.

11.0 APPENDIX B - PHARMACOLOGY/TOXICOLOGY

Pharmacology

Various studies were performed to characterize the pharmacology of trastuzumab (Herceptin®). These studies included both in vitro and in vivo assays. In vitro studies included trastuzumab-mediated HER2 receptor down modulation, complement-dependent cytotoxicity, and antibody-dependent cell mediated cytotoxicity. In vivo assays included efficacy studies of trastuzumab in human breast and ovarian tumor xenografts, as well as combination efficacy studies with cytotoxic chemotherapeutic agents.

Down modulation of receptor-ligand complexes is thought to be a major means of attenuating receptor induced signaling in cancer. To understand the extent and kinetics of trastuzumab-induced HER2 down modulation, various studies were undertaken. Using SK-BR-3 cells, the relative expression of the HER2 receptor on the cell surface decreased 24% from approximately 2.2 million sites per cell to approximately 1.7 million sites per cell as compared to untreated controls following a 24 hour in vitro incubation with trastuzumab. Five days of exposure to trastuzumab resulted in a 57% decrease in HER2 expression on SK-BR-3 cells over that time period. In this experiment sites per cell decreased from approximately 1.9 million on untreated cells to approximately 0.8 million on the antibody-treated cells. HER2 expression on MCF7 cells was decreased as well, showing a 51% decrease from approximately 18,000 sites per cell to approximately 9,000 sites per cell as compared to untreated controls. These data are consistent with the notion that trastuzumab down modulates HER2 receptor and may be a critical component in the induction of the antiproliferative effect.

A study of complement-dependent cytotoxicity and antibody dependent cell mediated cytotoxicity was also performed. Immunochemical and functional analysis demonstrated that trastuzumab activated complement after binding its target antigen, p185HER2, on 5 of 6 breast adenocarcinoma cell lines. However, trastuzumab-induced complement-mediated tumor cell lysis was not observed; this may be due to the presence of membrane-associated complement regulatory proteins such as CD35 (complement receptor 1, CR1), CD55 (decay accelerating factor, DAF), or CD46 (membrane cofactor protein, MCP). Complement-activating ability of the antibody was further supported by the results from solid-phase binding assays. Upon binding of trastuzumab to immobilized purified extracellular domain of HER2, activation of the complement cascade reaction could be demonstrated.

In studies of ADCC, trastuzumab demonstrated ADCC activity towards HER2-over expressing cells. Data from these experiments demonstrated that trastuzumab-dependent ADCC occurred primarily through interactions with FcγRIII or CO16. FcγRIII is expressed primarily on natural killer (NK) cells and monocytes/macrophages. Opsonization of tumor cell targets with trastuzumab was required to demonstrate ADCC.

Various in vivo assays were performed to demonstrate the efficacy of trastuzumab. MCF7 (human breast CA) cells were injected subcutaneously (3.5×10^7 cells/animal) in the mid-back region of 3-month old female Swiss nude mice. Seven days prior to injection cells, all mice received 17b-estradiol subcutaneous in a biodegradable carrier-binder (1.7 mg estradiol/pelet) that was expected to last for the duration of the study. A period of 7 days elapsed to allow formation of tumor nodules. Animals were randomized into six uniform groups based on animal weight and tumor sized at the start of the experiment (n = 7/group). Trastuzumab was tested at total doses of 3, 10, 30, and 100 mg/kg. Control injections included human IgG1 (total dose 100 mg/kg) and murine monoclonal antibody (muMAb) 4D5 (25 mg/kg) a murine antibody similar to trastuzumab. The biologic products were administered in three equally divided doses as intraperitoneal injections on Days 1,5, and 9. Tumor size in treated animal was followed until Day 21.

Mean 21-day tumor size relative to control human IgG1 was reduced for muMAb 4D5 and at all doses of trastuzumab tested ($p<0.01$). The extent of tumor growth inhibition was directly related to the dose of trastuzumab ($p<0.001$), as evaluated by a two-tailed trend test with ordinal dose scaling. Average tumor growth in animals treated with muMAb 4D5 was significantly ($p<0.004$) reduced upon comparison to an approximately equivalent dose of trastuzumab.

To determine the efficacy of trastuzumab in nude mice bearing BT-474 xenografts, animals experiments were conducted. Trastuzumab doses ranging from 0.1 to 30 mg/kg were administered twice a week by intraperitoneal injection for 4 weeks. Five to nine animals were treated in each group. The control group was treated with a nonspecific human IgG at 1 mg/kg or 30 mg/kg using the same schedule. Treatment was started when xenografts reached a size of 0.2-0.3 cm (Day 7 to 10). Dose-dependent antitumor activity was observed with 0.1, 0.3 and 1 mg/kg trastuzumab treatment when compared to mice treated with control antibody. Antitumor activity appeared to plateau at doses above 1 mg/kg.

CaOV3-HER2 cell were injected subcutaneously (5×10^7 cells/animal) in the mid-back region of 3-month old female Swiss nude mice. Estrogen support was used, and animals were randomized to receive human IgG1 (100 mg/kg), muMAb 4D5 (25 mg/kg), or trastuzumab at doses of 3, 10, 30, and 100 mg/kg (n = 5/group). Antibody was administered given as intraperitoneal injections on Days 1, 5, and 9. Tumor nodules were monitored twice week for three weeks by serial micrometer measurements.

Average 21-day tumor size reactive to control human IgG1 was reduced at all doses of trastuzumab tested. At the highest dose of trastuzumab a 10-fold decrease in tumor size was observed ($p<0.0001$). Average tumor size in muMAb 4D5 treated animals was significantly less than control ($p = 0.01$), but the murine antibody did not inhibit growth as well as trastuzumab at the 100 mg/kg dose ($p<0.02$).

In vitro experiments with the human breast cancer cell line, SK-BR-3, were conducted combining trastuzumab and eight drugs representing seven different classes of cytotoxic chemotherapeutic. In cell culture studies, cisplatin thiotepa, and etoposide were found to be synergistic with trastuzumab. Additive interactions were observed with doxorubicin, paclitaxel, methotrexate, and vinblastine; 5-fluorouracil, was found to exhibit an antagonistic interaction with trastuzumab.

Since SK-BR-3 cells are not tumorigenic in athymic mice, parallel studies were conducted in vivo using MCF7 breast cancer xenografts testing the same trastuzumab/drug combinations. Due to the number of athymic mice required, it was not possible to comprehensively study the multiple drug effects model in vivo. Instead, cytotoxic drug doses were based on independent dose escalation studies and were at or near the MTD values reported in the literature for each drug.

Trastuzumab doses were chosen to achieve serum concentration of > 10 μg/ml in animals bearing HER2 over expressing xenografts of 50-500 mm^3. This concentration of trastuzumab is approximately 5X greater than that required to see maximal antiproliferative activity in vitro.

Statistically superior antitumor efficacy was observed with trastuzumab in combination with doxorubicin, paclitaxel, cyclophosphamide, methotrexate, etoposide, and vinblastine. For the drug 5-fluorouracil, which was antagonistic with trastuzumab in vitro, the combination in vivo was superior to trastuzumab alone but not to 5-fluorouracil alone.

To further assess the antitumor activity of trastuzumab with cytotoxic chemotherapy, an alternative experimental systems was explored using the human breast cancer cell line. A derivative of the BT-474 cell line which overexpresses HER2 was selected for study. In combination studies, treatment with trastuzumab and doxorubicin or paclitaxel resulted in greater inhibition of tumor growth than observed with any agent alone. The combination of paclitaxel and trastuzumab resulted in the highest tumor growth inhibition and had significantly superior complete tumor regression rate when compared to paclitaxel or trastuzumab alone.

Toxicology

The various toxicity studies were conducted to support the clinical development of trastuzumab. These studies included acute toxicity in mice and monkeys, multiple-dose toxicity studies in monkeys for 4, 12, or 26 weeks, and a variety of special toxicity studies. Special toxicity studies included in vitro tissue cross reactivity in human and monkey tissues, interaction studies of trastuzumab with chemotherapeutic agents in monkeys and mice, acute local tolerance studies of liquid and lyophilized trastuzumab formulations in rabbits; and in vitro hemolytic potential and blood compatibility of liquid and lyophilized trastuzumab. Additionally, reproductive toxicity and mutagenicity studies were conducted. Based on the results of these studies, trastuzumab was shown to be reasonably safe when tested using vitro and in vivo systems. Based on the results from these studies, trastuzumab showed to be safe and non-toxic when tested in vitro and in vivo systems

12.0 APPENDIX C - ABBREVIATIONS

AC	anthracycline plus cyclophosphamide chemotherapy regimen
ACH	Herceptin® plus AC
ADCC	antibody-dependent cell-mediated cytotoxicity
Am 2	Amendment 2 to the protocol H0648g pivotal study
BLA	Biologics License Application
CAD	coronary artery disease or atherosclerosis
Chemo	chemotherapy
CHF	congestive heart failure
cm	centimeter
CR	complete response
CRF	Case Report Form
CT	computed tomographic scan
CXR	chest X-ray
Dig.	digoxin
DOE	dyspnea on exertion
DVT	deep venous thrombosis
Echo	echocardiogram
ECG or EKG	electrocardiogram
EGFR	endothelial growth factor receptor
FISH	fluorescence *in situ* hybridization
H	Herceptin®
HER2	human epidermal growth factor receptor 2
HER2/neu	human epidermal growth factor receptor 2
HR	heart rate

ICU	intensive care unit
IHC	immunohistochemistry
JVD	jugular venous distension
JVP	jugular venous pressure
MoAb or MAb	monoclonal antibody
MRI	magnetic resonance imaging
NYHA class	New York Heart Association Classification for congestive heart failure
O2	oxygen therapy
PD	progressive disease
Plt	platelet count
PND	paroxysmal nocturnal dyspnea
PR	partial response
RBC	red blood cell count
rhuMAb HER2	recombinant humanized anti-p185^{HER2} monoclonal antibody = Herceptin®
RR	respiratory rate
SD	stable disease
SOB	shortness of breath
T	paclitaxel chemotherapy regimen
TH	Herceptin® plus T
VEGF	vascular endothelial growth factor
WBC	white blood cell count
XRT	radiation therapy

13.0 APPENDIX D - DEFINITIONS

Please note that in cases where the FDA definition differs from the sponsor definition both versions appear and are annotated accordingly.

Complete response	Disappearance of all radiographically and/or visually apparent tumor for a minimum period of 4 weeks. Skin and chest wall complete responses were to be confirmed by biopsy.
Partial Response	A reduction of at least 50% in the sum of the products of the perpendicular diameters of all measurable lesions for a minimum period of 4 weeks. No new lesions may have appeared, nor may any lesion have progressed in size.
Minor Response (Genentech definition)	A reduction of 25% to 49% in the sum of the products of the perpendicular diameters of all measurable lesions. No new lesions may have appeared, nor may any lesion have progressed in size.
Minor Response (FDA definition)	Not defined by the FDA
Stable Disease (Genentech definition)	No change of greater than 25% in the size of measurable lesions. No new lesions.
Stable Disease (FDA definition)	No decrease of greater than 50% or increase of greater than 25% in the size of measurable lesions. No new lesions may have appeared.
Progressive Disease	Objective evidence of an increase of 25% or more in any measurable lesion. Progressive disease also included those instances where new lesions appeared.
Time to progression	The time to disease progression is calculated from the date of enrollment to the date of documented disease progression.
Time to treatment failure	The time to treatment failure is calculated from the date of enrollment to the date of progressive

	disease, death, introduction of additional non-protocol defined anti-tumor therapy, or study discontinuation for any reason.
Overall response rate	The rate of complete and partial responses as confirmed by the independent REC; partial and complete response must be sustained for at least 4 weeks.
Duration of response	The response duration is measured from the time point at which the response is first noted to the time point at which progressive disease is first noted.
Survival	Calculated for all patients from the date of enrollment to the date of death.